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HIGH THROUGHPUT METHODS OF HLA TYPING

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CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent application Serial No. 60/172,768, filed on December 20, 1999, the teachings of which are herein incorporated by reference.

FIELD OF THE INVENTION

In general, this invention relates to typing and matching human leukocyte antigens or alleles of human leukocyte antigens and in particular, to high throughput screening methods of human leukocyte antigen matching or alleles of human leukocyte antigens.

BACKGROUND OF THE INVENTION

The human leukocyte antigen complex (also known as the major histocompatibility complex) spans approximately 3.5 million base pairs on the short arm of chromosome 6. It is divisible into 3 separate regions which contain the class I, the class II and the class III genes. In humans, the class I HLA complex is about 2000 kb long and contains about 20 genes. Within the class I region exist genes encoding the well characterized class I MHC molecules designated HLA-A, HLA-B and HLA-C. In addition, there are nonclassical class I genes that include HLA-E, HLA-F, HLA-G, HLA-H, HLA-J and HLA-X as well as a new family known as MIC. The class II region contains three genes known as the HLA-DP, HLA-DQ and HLA-DR loci. These genes encode the α and β chains of the classical class II MHC molecules designated HLA-DR, DP and DQ. In humans, nonclassical genes designated DM, DN and DO have also been identified within class II. The class III region contains a heterogeneous collection of more than 36 genes. Several complete components are encoded by three genes including TNF-α and TNF-β.

Any given copy of chromosome 6 can contain many different alternative versions of each of the preceding genes and thus can yield proteins with distinctly different sequences. The loci constituting the MHC are highly polymorphic, that is, many forms of the gene or alleles exist at each locus. Several hundred different allelic variants of class I and class II MHC molecules have been identified in humans. However, any one

individual only expresses up to 6 different class I molecules and up to 12 different class II molecules.

The foregoing regions play a major role in determining whether transplanted tissue will be accepted as self (histocompatible) or rejected as foreign (histoincompatible). For instance, within the class II region, three loci *i.e.*, HLA-DR, DQ and DP are known to express functional products. Pairs of A and B genes within these three loci encode heterodimeric protein products which are multi-allelic and alloreactive. In addition, combinations of epitopes on DR and/or DQ molecules are recognized by alloreactive T cells. This reactivity has been used to define "Dw" types by cellular assays based upon the mixed lymphocyte reaction (MLR). It has been demonstrated that matching of donor and recipient HLA-DR and DQ alleles prior to allogeneic transplantation has an important influence on allograft survival. Therefore, HLA-DR and DQ matching is now generally undertaken as a clinical prerequisite for renal and bone marrow transplantation as well as cord blood applications.

Until recently, matching has been confined to serological and cellular typing. For instance, in the microcytotoxicity test, white blood cells from the potential donor and recipient are distributed in a microtiter plate and monoclonal antibodies specific for class I and class II MHC alleles are added to different wells. Thereafter, complement is added to the wells and cytotoxicity is assessed by uptake or exclusion to various dyes by the cells. If the white blood cells express the MHC allele for a particular monoclonal antibody, then the cells will be lysed on addition of complement and these dead cells will take up the dye. (*see*, Terasaki and McClelland, (1964) *Nature*, 204:998). However, serological typing is frequently problematic, due to the availability and crossreactivity of alloantisera and because live cells are required. A high degree of error and variability is also inherent in serological typing, which ultimately affects transplant outcome and survival (Sasazuki *et al.*, (1998) *New England J. of Medicine* 339: 1177-1185). Therefore, DNA typing is becoming more widely used as an adjunct, or alternative, to serological tests.

Initially, the most extensively employed DNA typing method for the identification of these alleles has been restriction fragment length polymorphism (RFLP) analysis. This well established method for HLA class II DNA typing suffers from a number of inherent drawbacks. RFLP typing is too time-consuming for clinical use prior to cadaveric renal transplantation for example, and for this reason it is best suited to live donor transplantation or retrospective studies. Furthermore, RFLP does not generally

detect polymorphism within the exons which encode functionally significant HLA class II epitopes, but relies upon the strong linkage between alleles-specific nucleotide sequences within these exons and restriction endonuclease recognition site distribution within surrounding, generally noncoding, DNA.

In addition to restriction fragment length polymorphism (PCR-RFLP), an even more popular approach has been the hybridization of PCR amplified products with sequence-specific oligonucleotide probes (PCR-SSO) to distinguish between HLA alleles (*see*, Tiercy *et al.*, (1990) *Blood Review* 4: 9-15). This method requires a PCR product of the HLA locus of interest be produced and then dotted onto nitrocellulose membranes or strips. Then each membrane is hybridized with a sequence specific probe, washed, and then analyzed by exposure to x-ray film or by colorimetric assay depending on the method of detection. Similar to the PCR-SSP methodology, probes are made to the allelic polymorphic area responsible for the different HLA alleles. Each sample must be hybridized and probed at least 100-200 different times for a complete Class I and II typing. Hybridization and detection methods for PCR-SSO typing include the use of non-radioactive labeled probes, microplate formats, etc. (see e.g., Saiki *et al.* (1989) *Proc. Natl. Acad. Sci., U.S.A.* 86: 6230-6234; Erlich *et al.* (1991) *Eur. J. Immunogenet.* 18(1-2): 33-55; Kawasaki *et al.* (1993) *Methods Enzymol.* 218:369-381), and automated large scale HLA class II typing. A common drawback to these methods, however, is the relatively long assay times needed--generally one to two days--and their relatively high complexity and resulting high cost. In addition, the necessity for sample transfers and washing steps increases the chances that small amounts of amplified DNA might be carried over between samples, creating the risk of false positives.

More recently, a molecular typing method using sequence specific primer amplification (PCR-SSP) has been described (*see*, Olerup and Zetterquist (1992) *Tissue Antigens* 39: 225-235). This PCR-SSP method is simple, useful and fast relative to PCR-SSO, since the detection step is much simpler. In PCR-SSP, allelic sequence specific primers amplify only the complementary template allele, allowing genetic variability to be detected with a high degree of resolution. This method allows determination of HLA type simply by whether or not amplification products (collectively called an "amplicon") are present or absent following PCR. In PCR-SSP, detection of the amplification products is usually done by agarose gel electrophoresis followed by ethidium bromide (EtBr) staining of the gel. Unfortunately, the electrophoresis process takes a long time and is not very suitable for large number of samples, which is a problem since each

clinical sample requires testing for many potential alleles. Gel electrophoresis also is not easily adapted for automatic HLA-DNA typing.

Another HLA typing method is SSCP - Single-Stranded Conformational Polymorphism. Briefly, single stranded PCR products of the different HLA loci are run on non-denaturing Polyacrylamide Gel Electrophoresis (PAGE). The single strands will migrate to a unique location based on their base pair composition. By comparison with known standards, a typing can be deduced. It is the only method that can determine true homozygosity. However, many PAGE have to be run and many controls have to be run to make it a viable typing method. This method is very time consuming, labor intensive, and not really suited for large volume analysis.

In view of the foregoing, what is needed in the art is a method of determining genomic information from a highly polymorphic system such as the HLA class I and class II regions. The present invention provides a highly accurate and efficient HLA class I and class II sequence-based typing method that is rapid, reliable and completely automatable.

SUMMARY OF THE INVENTION

The present invention provides new and improved methods for HLA typing. In addition, the methods eliminate the reliance on agarose gel electrophoresis usage for the sequence specific primer (SSP) method for performing HLA DNA typing and obviates the reliance on using cumbersome blot membranes for sequence-specific oligonucleotide probe hybridization (SSO) as well as many of the human errors associated with manual interpretation of bands and assignment of alleles. Thus, the methods of the present invention decrease significantly the number of human errors and the amount of time and effort it takes to perform DNA HLA typing.

In certain aspects, the present invention provides a method of detecting amplified DNA in which the risks of sample cross-contamination and resulting false positive results are reduced. In addition, the present invention provides methods that can allow for reliable, rapid analysis of multiple samples. Moreover, the present invention provides a method of detecting amplified DNA that is relatively simple, and results in a relatively low cost per analysis and is amenable to automation and high throughput matching.

In one aspect, the present invention provides methods for identifying an HLA genotype of a subject. The method involves (a) obtaining a sample containing a template nucleic acid from said subject; (b) amplifying the template nucleic acid with a

plurality of HLA allele-specific forward primers and HLA allele-specific reverse primers to form amplification products, wherein the forward primers or reverse primers comprise a detectable label; (c) hybridizing the amplification products with a plurality of HLA locus-specific capture oligonucleotides immobilized on a solid phase to form a plurality of detectable complexes; and (d) detecting the detectable complexes to identify the HLA genotype of the subject.

Another aspect of the present invention provides methods for identifying an HLA genotype of a subject that involves (a) obtaining a sample containing a template nucleic acid from the subject; (b) amplifying the template nucleic acid with a plurality of HLA allele-specific forward primers and HLA allele-specific reverse primers to form amplification products, wherein the forward primers or reverse primers contain a detectable label; (c) hybridizing the amplification products with a plurality of HLA locus-specific capture oligonucleotides to form a plurality of detectable complexes; (d) immobilizing the detectable complexes on a solid phase; and (e) detecting the detectable complexes to identify the HLA genotype of the subject.

In yet another aspect of the invention, methods for identifying an HLA genotype of a subject is provided that involves: immobilizing a plurality of HLA allele-specific reverse primers on a solid phase; amplifying the template nucleic acid with a plurality of HLA allele-specific forward primers and the immobilized reverse HLA allele-specific reverse primers to form amplification products; and detecting the amplification products to identify the HLA genotype of the subject.

In certain embodiments of the present invention, template nucleic acid that is isolated from blood or cord blood is amplified. The template nucleic acid can be any gene derived sequences, including, but not limited to cDNA and genomic DNA.

In certain embodiments, oligonucleotides are immobilized on a solid phase. Examples of solid phase include, but are not limited to: a bead, a chip, a microtiter plate, a polycarbonate microtiter plate, polystyrene microtiter plate, and a slide.
The methods of the present invention can be also used to determine class I and class II HLA genotypes. In certain embodiments, HLA allele-specific forward primers and HLA allele-specific reverse primers are used to amplify the template nucleic acid to generate amplification products. In some embodiments, the HLA allele-specific primers are selected from primers denoted as SEQ ID NOS:1-160 and
SEQ ID NOS: 169-269.

In some embodiments of the invention, capture oligonucleotides are employed. In certain preferred embodiments, locus-specific capture oligonucleotides are used in the HLA genotyping methods and can be selected from the primers such as SEQ ID NOS: 272-277 and SEQ ID NOS:165-168. The capture oligonucleotides can be modified with a moiety that aids in immobilizing the capture oligonucleotide to a solid phase. In certain embodiments, moieties such as a 5' amine group or a 5'$(T)_{5-20}$ oligonucleotide sequence are utilized.

Detectable labels can be used with certain embodiments of the present invention. Examples of a detectable label, include, but are not limited to a radioactive moiety, a fluorescent moiety, a chemiluminescent moiety, an antigen, or a binding protein. In certain embodiments, fluorescent moieties such as fluorescein or 5-(2'-aminoethyl) aminonaphtalene-1-sulfonic acid (EDANS) are attached to oligonucleotides to facilitate detection.

These embodiments as well as additional objects and advantages will become more readily apparent when read with the accompanying figure and detailed description which follows.

DEFINITIONS

An "allele" is one of the different nucleic acid sequences of a gene at a particular locus on a chromosome. One or more genetic differences can constitute an allele. Examples of HLA allele sequences are set out in Mason and Parham (1998) *Tissue Antigens* 51: 417-66, which list HLA-A, HLA-B, and HLA-C alleles and Marsh *et al.* (1992) *Hum. Immunol.* 35:1, which list HLA Class II alleles for DRA, DRB, DQA1, DQB1, DPA1, and DPB1.

A "locus" is a discrete location on a chromosome that constitutes a gene. Exemplary loci are the class I MHC genes designated HLA-A, HLA-B and HLA-C; nonclassical class I genes including HLA-E, HLA-F, HLA-G, HLA-H, HLA-J and HLA-X, MIC; and class II genes such as HLA-DP, HLA-DQ and HLA-DR.

A method of "identifying an HLA genotype" is a method that permits the determination or assignment of one or more genetically distinct HLA genetic polymorphisms.

The term "amplifying" refers to a reaction wherein the template nucleic acid, or portions thereof, are duplicated at least once. Unless specifically stated "amplifying" may refer to arithmetic, logarithmic, or exponential amplification. The

amplification of a nucleic acid can take place using any nucleic acid amplification system, both isothermal and thermal gradient based, including but not limited to, polymerase chain reaction (PCR), reverse-transcription-polymerase chain reaction (RT-PCR), ligase chain reaction (LCR), self-sustained sequence reaction (3SR), and transcription mediated amplifications (TMA). Typical nucleic acid amplification mixtures (*e.g.*, PCR reaction mixture) include a nucleic acid template that is to be amplified, a nucleic acid polymerase, nucleic acid primer sequence(s), and nucleotide triphosphates, and a buffer containing all of the ion species required for the amplification reaction.

An "amplification product" is a single stranded or double stranded DNA or RNA or any other nucleic acid products of isothermal and thermal gradient amplification reactions that include PCR, TMA, 3SR, LCR, etc.

The phrase "template nucleic acid" refers to a nucleic acid polymer that is sought to be copied or amplified. The "template nucleic acid(s)" can be isolated or purified from a cell, tissue, animal, etc. Alternatively, the "template nucleic acid(s)" can be contained in a lysate of a cell, tissue, animal, etc. The template nucleic acid can contain genomic DNA, cDNA, plasmid DNA, etc.

An "HLA allele-specific" primer is an oligonucleotide that hybridizes to nucleic acid sequence variations that define or partially define that particular HLA allele.

An "HLA locus-specific" primer is an oligonucleotide that permits the amplification of a HLA locus sequence or that can hybridize specifically to an HLA locus.

A "forward primer" and a "reverse primer" constitute a pair of primers that can bind to a template nucleic acid and under proper amplification conditions produce an amplification product. If the forward primer is binding to the sense strand then the reverse primer is binding to antisense strand. Alternatively, if the forward primer is binding to the antisense strand then the reverse primer is binding to sense strand. In essence, the forward or reverse primer can bind to either strand as long as the other reverse or forward primer binds to the opposite strand.

The term "detectable label" refers to a moiety that is attached through covalent or non-covalent means to an oligonucleotide. A "detectable label" can be a radioactive moiety, a fluorescent moiety, a chemiluminescent moiety, etc.

The term "fluorescent label" refers to label that accepts radiant energy of one wavelength and emits radiant energy of a second wavelength.

The phrase "hybridizing" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence or subsequence through specific binding of two nucleic acids through complementary base pairing. Hybridization typically involves the formation of hydrogen bonds between nucleotides in one nucleic acid and complementary sequences in the second nucleic acid.

The phrase "hybridizing specifically" refers to hybridizing that is carried out under stringent conditions.

The term "stringent conditions" refers to conditions under which a capture oligonucleotide, oligonucleotide or amplification product will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the capture oligonucleotides are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is at most about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. An extensive guide to the hybridization and washing of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in biochemistry and molecular biology--hybridization with nucleic acid probes parts I and II,* Elsevier, New York, and, Choo (ed) (1994) *Methods In Molecular Biology Volume 33- In Situ Hybridization Protocols Humana Press Inc.*, New Jersey; Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., (1994)).

The term "complementary base pair" refers to a pair of bases (nucleotides) each in a separate nucleic acid in which each base of the pair is hydrogen

bonded to the other. A "classical" (Watson-Crick) base pair always contains one purine and one pyrimidine; adenine pairs specifically with thymine (A-T), guanine with cytosine (G-C), uracil with adenine (U-A). The two bases in a classical base pair are said to be complementary to each other.

"Bind(s) substantially" refers to complementary hybridization between a capture nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence.

The term "capture oligonucleotide" refers to a nucleic acid sequence or nucleic acid subsequence that can hybridize to another oligonucleotide, amplification product, etc. and has the ability to be immobilized to a solid phase. A capture oligonucleotide typically hybridizes to at least a portion of an amplification product containing complementary sequences under stringent conditions.

A "HLA locus-specific capture oligonucleotide" is a capture oligonucleotide that is complementary to and hybridizes to a conserved region of an HLA locus. For example a "HLA locus-specific capture oligonucleotide" that is specific for the HLA-A locus will hybridize to one or more conserved regions or subsequences of the HLA-A locus.

A compound is "immobilized on a solid phase" when it is directly or indirectly attached to the solid phase. Such immobilization may be through covalent and/or non-covalent bonds.

The term "corresponding nucleotide," is used to refer to the position of a nucleotide in a first nucleic acid by reference to a second nucleic acid. Thus, a corresponding nucleotide refers to a nucleotide that it is positionally located opposite to a base where neighboring bases are all hybridized pairs.

"Subsequence" refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

The term "portions" should similarly be viewed broadly, and would include the case where a "portion" of a DNA strand is in fact the entire strand.

The term "specificity" refers to the proportion of negative test results that are true negative test result. Negative test results include false positives and true negative test results.

The term "sensitivity" is meant to refer to the ability of an analytical method to detect small amounts of analyte. Thus, as used here, a more sensitive method for the detection of amplified DNA, for example, would be better able to detect small amounts of such DNA than would a less sensitive method. "Sensitivity" refers to the proportion of expected results that have a positive test result.

The term "reproducibility" as used herein refers to the general ability of an analytical procedure to give the same result when carried out repeatedly on aliquots of the same sample.

The term "amplicon" is used herein to mean a population of DNA molecules that has been produced by amplification, *e.g.*, by PCR.

The term "molecular beacon," as used herein refers to a molecule capable of participating in a specific binding reaction and whose fluorescence activity changes when the molecule participates in that binding reaction.

DETAILED DESCRIPTION

I. INTRODUCTION

The present invention provides methods for HLA genotyping of human leukocyte antigens, as well as other molecular diagnostic protocols relating to the detection of DNA sequences and sequence variations using nucleic acid amplification methods. Advantageously, the methods described herein can be used to detect genetic mutations, detect cancer gene mutations, microbial and cancer drug resistance mutations, detection of viruses, bacteria, fungi, parasites and any other microbes, forensics, parentage, *etc.*

In particular, the methods of the present invention are useful for determining HLA genotypes of samples from subjects. Such genotyping is important in the clinical arena for the diagnosis of disease, transplantation of organs, and bone marrow and cord blood applications.

In the present invention, allelic-specific HLA primers are used to amplify HLA sequences. In some embodiments, these amplification products can be immobilized to a solid phase using a locus-specific or an allele-specific capture oligonucleotide. In certain embodiments, the locus-specific capture oligonucleotides are preferred as fewer capture oligonucleotides need to be generated to carry out the HLA genotyping. In other embodiments, one HLA-specific primer is immobilized to a solid phase and the target is

amplified using another HLA-specific primer that is free in solution. The advantages and details for carrying out the present invention will be discussed more fully below.

II. MATERIALS USED IN THE PRESENT INVENTION

Oligonucleotides

Oligonucleotides used in the present invention (e.g., allele and locus-specific oligonucleotides) can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, (1981) *Tetrahedron Letts.* 22:1859-1862, using an automated synthesizer, as described in Van Devanter *et al.,* (1984) *Nucleic Acids Res.* 12: 6159-6168. Purification of oligonucleotides is typically by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, (1983) *J. Chrom.* 255:137-149.

HLA allele-specific primers

The HLA allele-specific primers used in the present invention are designed to amplify HLA allele sequences. Since 1995, 213 class I (HLA-A, HLA-B, and HLA-C) and 256 class II (HLA-DR, HLA-DP, and HLA-DQ) alleles had been identified and sequenced (see e.g., Krausa and Browning (1996) *Detection of HLA gene polymorphism* in Browning M, McMichael A, ed. *HLA and MHC: genes, molecules and function.* Oxford: Bios Scientific Publishers Limited, pp. 113-138), with new alleles being discovered all the time. The sequences of many of these alleles are publicly available through GenBank and other gene databases and have been published (see e.g., Mason and Parham (1998) *Tissue Antigens* 51: 417-66, listing HLA-A, HLA-B, and HLA-C alleles; Marsh *et al.* (1992) *Hum. Immunol.* 35:1, listing HLA Class II alleles – DRA, DRB, DQA1, DQB1, DPA1, and DPB1). Also, the use of allele-specific primers (sequence-specific primers (SSP)) has permitted the specific amplification of HLA allele sequences (see e.g., Bunce and Welsh (1994) *Tissue Antigens* 43: 7-17, amplification of HLA-C alleles; Bunce *et al.* (1995) *Tissue Antigens* 46: 355-67, amplification of HLA-A.B.C. DRB1, DRB3, DRB4, DRB5 & DQB1 alleles with sequence-specific primers; Gilchrist *et al.* (1998) *Tissue Antigens* 51: 51-61, HLA-DP typing with sequence specific primers).

In the design of the HLA primer pairs for the primer mixes, primers were selected based on the published HLA sequences available in the literature. A chart of the HLA alleles and sequences was examined and the polymorphic sites were identified. Then pairs of primers were selected that would produce PCR products to a group of HLA alleles. The sequence specific nucleic acid amplification reaction typically uses at least a

pair of PCR primers for each allele, both of which contain the discriminating sequences with at least one or more of the changed nucleotides at the 3' end of each PCR primer. Since the 3' end is the end where polymerization takes place, if a mismatch occurs due to sequence non-complementarily, nucleic acid amplification will take place and one would not expect a "false positive." However, if a match occurs, then the amplification can proceed. For example, HLA class I allele-specific primers and HLA class II allele-specific primers are listed in Table 1 (SEQ ID NOS: 1-160) and 2 (SEQ ID NOS: 169-269), respectively. Examples of control primers listed in Table 1 are CI53 (SEQ ID NO: 161), CI54 (SEQ ID NO: 162), CI148 (SEQ ID NO: 163), and CI149 (SEQ ID NO: 164). Examples of control primers listed in Table 2 are DPA-E(PC) (SEQ ID NO: 270), and DPA-F (PC) (SEQ ID NO: 271). The Class I primers are selected to amplify Class I exon 2 and exon 3 products. The Class II primers are selected to amplify Class II exon 2 products. In certain embodiments, the primers listed in Tables 3 and 4 are used as exemplary groups of primer pairs and the HLA specificities these pairs can identify after successful positive PCR amplifications with the appropriate DNA templates for HLA class I and II alleles respectively. By utilizing a pair of primers, each PCR reaction identifies two sites of polymorphism and therefore increases the specificity of the reaction. Those of skill in the art will recognize a multitude of oligonucleotide compositions that can be used as HLA allele-specific primers to specifically amplify HLA allele sequences. In addition, customized sets of HLA-specific primers can be created to cater to detection of the allele distribution for various ethnicities or racial groups by simply changing the primer pair combinations. In this manner, detection of new alleles can be easily added to the methods of the present invention.

Capture Oligonucleotides

In certain embodiments, the invention involves locus-specific capture oligonucleotides or allele-specific capture oligonucleotides. Locus-specific oligonucleotide can hybridize to a conserved region in a HLA locus; a locus-specific capture oligonucleotide has the ability to hybridize to some or all of the sequences that can be generated by the amplification of HLA allele sequences using HLA-specific primers. Locus-specific sequences have been identified in HLA loci. For example, locus-specific sequences for HLA-class I genes have been delineated in the first and third introns flanking the polymorphic second and third exons (see e.g., Cereb *et al.* (1995) *Tissue Antigens* 45: 1-11). The capture oligonucleotides should be of such length and composition so as to be able to hybridize with the allele-specific PCR products. In certain

embodiments, HLA locus-specific class I capture oligonucleotides contain the following sequences: for HLA-A (CICptA1, Class I Capture Oligo A1, 5'ACGCCTACGACGGCAAGGATTACATCGCCC3' (SEQ ID NO:165); and CICptA2, Class I Capture Oligo A2, 5'GATGGAGCCGCGGTGGATAGAGCAGGAGGG3'(SEQ ID NO:166), for HLA-B (CICptB1, Class I Capture Oligo B1, 5'CAGTTCGTGAGGTTCGACAGCGACGCC3'(SEQ ID NO:167), and CICptB2, Class I Capture Oligo B2, 5'CTGCGCGGCTACTACAACCAGAGCGAGGCC3'(SEQ ID NO:168). In other embodiments, HLA locus-specific class II capture oligonucleotides contain the following sequences:

for HLA-DQ (DQCPT1, 5'CACGTCGCTGTCGAAGCGCACGTACTCCTC3' (SEQ ID NO:272); DQCPT2, 5'CACGTCGCTGTCGAAGCGGACGATCTCCTT3' (SEQ ID NO:273); DQCPT3, 5'CACGTCGCTGTCGAAGCGTGCGTACTCCTC3' (SEQ ID NO:274); DQCPT4, 5'CACGTCGCTGTCGAAGCGCGCGTACTCCTC3' (SEQ ID NO:275); and

DQCPT5, 5'CACGTCGCTGTCGAAGCGCACGTCCTCCTC3'(SEQ ID NO:276), for HLA-DR (DRCPT1, DRCP, 5'TGGCGTGGGCGAGGCAGGGTAACTTCTTTA3' (SEQ ID NO:277)). In certain embodiments, it may require the use of more than one capture oligonucleotide to hybridize to all of the HLA allele amplification products.

Modification of Oligonucleotides

In certain embodiments of the present invention, oligonucleotides are modified or synthesized as modified oligonucleotides to facilitate immobilization or detection.

Immobilization modifications

In certain embodiments, where capture oligonucleotides are used or where an immobilized amplification primer is used, it is desirable to modify the particular oligonucleotide to affix it to a solid phase or support. It is desired that the modification of the capture oligonucleotide does not interfere with its ability to bind to an HLA allele-specific amplification product. Those of skill in the art will recognize a variety of methods to immobilize oligonucleotides to a solid phase. For example, oligonucleotides can be directly or indirectly immobilized on a solid phase. The oligonucleotides can be immobilized directly to the solid phase through covalent and non-covalent bonds. For example, the 5' end of an oligonucleotide can be synthesized with an amine moiety (see Kawasaki *et al.* (1993)). In certain embodiments, an amine moiety with a C6 carbon spacer is conjugated to the 5' end of a capture oligo or amplification primer. The amine-

modified primers are affixed to the surface of a substrate such a Biodyne C membrane (Pall Biosupport) (Kawasaki *et al.* (1993)) or through a commercially available microtiter plate (e.g., Xenobind™ (Covalent Binding Microwell Plates), Xenopore, Hawthorne, NJ). Alternatively a polythymidine (polyT) stretch can be added to an oligonucleotide by terminal deoxyribonucletotidyltransferase (Saiki *et al.* (1989)). Such a polyT stretch can be fixed to many solid substrates (e.g., nylon) using UV light leaving the rest of the oligonucleotide free to hybridize to another nucleic acid. Preferably, the polyT stretch is from 5 to 20 T's.

Alternatively, the oligonucleotides can be indirectly bound to the solid phase by coating the solid phase with a substance or molecule that can bind to the oligonucleotides. Biotinylated oligonucleotides can also be used as capture oligonucleotides. Methods are known in the art for synthesizing biotinylated oligonucleotide (e.g., by synthesizing a primer with a biotinylated 5' end nucleotide as the terminal residue) (see e.g., Innis *et al.* (1990)). Biotinylated oligonucleotides can be affixed to a substrate that is coated with avidin.

A high density array of capture oligonucleotides or amplification primers can be also synthesized on a substrate by attaching photoremovable groups to the surface of a substrate, exposing selected regions of the substrate to light to activate those regions, attaching a nucleic acid monomer with a photoremovable group to the activated regions, and repeating the steps of activation and attachment until probes of the desired length and sequences are synthesized. (See, *e.g.*, Fodor *et al.* (1991) *Science* 251: 767-773 and U.S. Patent No. 5,143,854). The resulting array of oligonucleotides can then be used to in the methods of the present invention.

A variety of solid supports or phases can be used in the present invention. Examples of solid supports include, without limitation, bead, microtiter plates, and chips. Beads can be composed of materials such as Sepharose, agarose, polystyrene, etc. and can be paramagnetic. Microtiter plates are commercially available in a variety of formats (e.g., 96, 384 and 1536 well plates) and materials (e.g., polystyrene). The plates can be either polycarbonate plates in which case the thermal gradient nucleic acid amplification reaction (such as PCR) can happen directly in the well or polystyrene in which case the thermal gradient nucleic acid amplification reaction (such as PCR) has to take place in a separate polycarbonate plate and transferred to the surface modified and oligonucleotide attached plate. Isothermal nucleic acid amplification methods can be conducted in

polystyrene plates. chips can be comprised of a variety of materials, layers and substrates. Polymers which may be used as solid supports or phases include, but are not limited to, the following: polystyrene; poly(tetra)fluoroethylene (PTFE); polyvinylidenedifluoride; polycarbonate; polymethylmethacrylate; polyvinylethylene; polyethyleneimine; poly(etherether)ketone; polyoxymethylene (POM); polyvinylphenol; polylactides; polymethacrylimide (PMI); polyatkenesulfone (PAS); polypropylene; polyethylene; polyhydroxyethylmethacrylate (HEMA); polydimethylsiloxane; polyacrylamide; polyimide; and block-copolymers. The solid support on which an oligonucleotide resides may also be a combination of any of the aforementioned solid support materials.

Oligonucleotides Containing Detectable Labels

Detectable labels can also be attached to oligonucleotides to facilitate detection of the oligonucleotide in an analyte. Detectable labels can be detected either directly or indirectly, by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radiolabels (e.g., ^{3}H, ^{13}C, ^{14}C, ^{32}P, ^{35}S, ^{125}I, etc.), fluorescent dyes, fluorophores, fluorescent moieties, chemiluminescent moieties, electron-dense reagents, enzymes and their substrates (*e.g.*, as commonly used in enzyme-linked immunoassays, *e.g.*, alkaline phosphatase and horse radish peroxidase), biotin-streptavidin, digoxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available. The label or detectable moiety is typically bound, either covalently, through a linker or chemical bound, or through ionic, van der Waals or hydrogen bonds to the molecule to be detected.

The detectable label should be stable to the amplifications conditions used and should permit direct or indirect detection. Indirect detection often involves the presence of one or more detection reagents. For example, one detectable label, biotin can be detected using an avidin conjugate such as avidin conjugated to an enzyme such as peroxidase (e.g., HRP), and a colorimetric substrate for peroxidase (e.g., TMB). The formation of colorimetric product can easily be detected using a spectrophotometer. For example, in certain embodiments, the primers listed in Tables 5 and 6 are biotinylated.

In certain embodiments, oligonucleotides comprising a quencher and a fluorophore moiety (molecular beacons) are contemplated. Molecular Beacons are single stranded oligonucleotide probes designed to have hairpin configuration by virtue of the presence of five to seven complementary nucleotides at their termini. The loop portion (10-40 nucleotides) is chosen so that the probe-amplification product hybrid is stable at

the annealing temperature. The length of the arm sequences (5-7 nucleotides) is chosen so that a stem is formed at the annealing temperature of the polymerase chain reaction. Also the stem or arm sequence must be designed to ensure that the two arms hybridize to each other but not to the probe sequence. One end would carry a fluorophore (e.g. 5-(2'-aminoethyl) aminonaphtalene-1-sulfonic acid (EDANS) and the other a quencher (e.g. 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL). When a probe is not hybridized to its complementary target sequence, the hairpin folding reaction would take place and fluorescence does not occur due to quenching. Quenching occurs because the energy given off as light during fluorescence is transferred to the quencher and dissipated as heat. Since the energy is released as heat instead of light, the fluorescence is said to be quenched. However, if a complementary target sequence is present, hybridization to the target sequence would be favored over the internal hairpin due to the increased stability as a result of the longer stretches of complementary sequence. The hairpin would open up thus allowing for release of quenching and the probes to fluoresce. In the fluorophore-quencher pair example given above, when stimulated by UV light with a peak wavelength of 336 nm, EDANS emits a brilliant blue fluorescence with a peak wavelength of 490 nm. (Tyagi *et al.*, (1996) *Nature Biotechnology* 14: 303-308; Tyagi *et al.* (1998) *Nature Biotechnology* 16:49-53; Paitek *et al.* (1998) *Nature Biotechnology* 16: 359-63; Kostrikis *et al.* (1998) *Science* 279:1228-1229).

III. SOURCE OF HLA GENE SEQUENCES

The template HLA DNA sequences are contained in samples containing nucleic acid (e.g., DNA, RNA, etc.), which are obtained from a biological source. In certain embodiments, the nucleic acid is isolated from a biological source containing HLA gene sequences. The nucleic acid may be from any species having HLA gene sequences, which include but are not limited to, a human, a chimpanzee, a simian, a mouse, etc. Methods are known for lysing biological samples and preparing extracts or purifying DNA, RNA, etc. See, *Current Protocols in Molecular Biology* (Ausubel et al., eds., 1994)). In some embodiments, the biological source is blood, and is more preferably cord blood (e.g., blood from an umbilical cord). In methods involving cord blood or blood, two isolation procedures are preferred: Salt extraction with ethanol precipitation; and the Qiagen QIAamp® isolation method. For the salt extraction method, the cells are first lysed and centrifuged. Then water is added and the sample is centrifuged again. The pellet is digested with Proteinase K. The DNA is then extracted

by the addition of 6M Guanidine HCl and incubation at 70°C for several minutes. The sample is centrifuged again and the supernatant is precipitated with cold 95% Ethanol. The pellet is then dried and resuspended in the appropriate buffer.

RNA template sequences that are amplified using the methods and compositions of the present invention may be a single RNA template or different RNA templates. The RNA can be isolated as total RNA from a cell, bacterium, virus etc. See, Ausubel *et al.* The total RNA may be subsequently purified as poly A+ RNA or purified in a different manner to isolate certain species of interest. See Ausubel *et al.* Alternatively, the template RNA can be transcribed *in vitro* and used in the present invention. The RNA template sequence could also be reverse transcribed into cDNA and used as a nucleic acid template in the methods of the present invention.

IV. AMPLIFICATION OF HLA GENE SEQUENCES FROM NUCLEIC ACID

The methods of the present invention involve the direct or indirect detection of HLA gene sequences that have been amplified from DNA or reverse transcribed DNA. To amplify the desired nucleic acid for HLA gene sequences, the following are usually present in the reaction vessel: template nucleic acid, nucleic acid polymerase, a molar excess of dNTPs, an antisense primer(s), and a sense-primer(s), for copying a HLA gene sequence from a template nucleic acid. Preferably, the reaction can be carried out in a thermal cycler oven to facilitate incubation times at the desired temperatures.

Reaction components

Oligonucleotide Primers

The oligonucleotides that are used in the present invention, as well as oligonucleotides designed to detect amplification products, can be chemically synthesized as described above. These oligonucleotides can be labeled with radioisotopes, chemiluminescent moieties, or fluorescent moieties, etc. in a covalent or non-covalent manner. Such labels are useful for the characterization and detection of amplification products using the methods and compositions of the present invention.

Buffer

Buffers that may be employed are borate, phosphate, carbonate, barbital, Tris, etc. based buffers. See Rose *et al.*, U.S. Patent No. 5,508,178. The pH of the reaction should be maintained in the range of about 4.5 to about 9.5. See U.S. Patent No. 5,508,178. The standard buffer used in amplification reactions is a Tris based buffer

between 10 and 50 mM with a pH of around 8.3 to 8.8. See Innis *et al.* (1990). In certain embodiments of the invention, a preferred buffer for the present invention is 150 mM Tris-HCl pH 8.8 for the amplification of class I HLA sequences and 20 mM Tris HCl pH 8.8 for class II HLA sequences.

Salt concentration

The concentration of salt present in the reaction can affect the ability of primers to anneal to the template nucleic acid. See Innis *et al.* (1990). For example, potassium chloride can be added up to a concentration of about 50 mM to the reaction mixture to promote primer annealing. Sodium chloride can also be added to promote primer annealing. See Innis *et al.* (1990). In certain embodiments of the invention, the preferred salts are 30 mM Ammonium Chloride for class I HLA sequences and 100 mM KCl for class II sequences.

Magnesium ion concentration

The concentration of magnesium ion in the reaction can be critical to amplifying the desired sequence(s). See Innis *et al.* (1990). Primer annealing, strand denaturation, amplification specificity, primer-dimer formation, and enzyme activity are all examples of parameters that are affected by magnesium concentration. See Innis *et al.* (1990). Amplification reactions should contain about a 0.5 to about a 5 mM magnesium concentration excess over the concentration of dNTPs. The presence of magnesium chelators in the reaction can affect the optimal magnesium concentration. A series of amplification reactions can be carried out over a range of magnesium concentrations to determine the optimal magnesium concentration. The optimal magnesium concentration can vary depending on the nature of the template nucleic acid(s) and the primers being used, among other parameters. In certain embodiments of the invention, the preferred magnesium concentrations are 4 mM $MgCl_2$ and 3.4 mM $MgCl_2$, for class I HLA sequences and class II HLA sequences, respectively.

Deoxyribonucleotide Triphosphate concentration

Deoxyribonucleotide triphosphates (dNTPs) are added to the reaction to a final concentration of about 20 μM to about 300 μM. Each of the four dNTPs (G, A, C, T) should be present at equivalent concentrations. See Innis *et al.* In certain embodiments, 166 μM dNTP is the preferred concentration of nucleotides.

Nucleic acid polymerase

A variety of DNA dependent polymerases are commercially available that will function using the methods and compositions of the present invention. For example, *Taq* DNA Polymerase may be used to amplify template DNA sequences. Also, a reverse transcriptase can be used in certain embodiments of the present invention. Reverse transcriptases, such as the thermostable *C. therm* polymerase from Roche, are also widely available on a commercial basis.

Other agents

Assorted other agents or compounds are sometime added to the reaction to achieve the desired results. For example, DMSO can be added to the reaction, but is reported to inhibit the activity of *Taq* DNA Polymerase. However, DMSO has been recommended for the amplification of multiple template sequences in the same reaction. See Innis *et al.* Stabilizing agents such as gelatin, bovine serum albumin, and non-ionic detergents (*e.g.* Tween-20) are commonly added to amplification reactions. See Innis *et al.* For the amplification of class II sequences, the addition of 0.2% Triton X-100 has been found to be preferred. In addition, to enhance specificity by decreasing spurious priming, methods that incorporate "hot start" (e.g., AmpliWax® (Applied Biosystems, Inc.), or an monoclonal antibody to *Taq* polymerase (CLONTECH Laboratories, Inc.) can be used to increase the specificity of an amplification reaction.

Amplification Programs

To amplify the HLA gene sequences of interest, the amplification reaction mixture is subjected to a series of temperatures to repeatedly denature the nucleic acid, anneal the oligonucleotide primers, and extend the primers with the polymerase. The use of a thermal cycling device can greatly facilitate the temperature cycling required in certain embodiments of the present invention. The optimum denaturing, annealing and extending temperatures can be determined by one of skill in the art for a particular oligonucleotide primer pair(s) and HLA gene template(s). In general, the extension step is carried out at a temperature of about 72 °C and the denaturing step is carried out at about 96 °C. In addition, it may be necessary to carry out different sets of amplification cycles in succession to achieve the desired results. In addition, the number of cycles is an important consideration. Typically, one of skill in the art can carry out experiments to determine what is the optimum number of cycles to amplify the desired template(s).

The annealing temperature is of critical importance in any amplification reaction. If the annealing temperature is too low, non-specific amplification of undesired templates can arise. If the annealing temperature is too high, the template may not be efficiently amplified if at all. Determining the optimum annealing temperature for in reactions that involve large numbers of different oligonucleotide sequences and HLA templates is particularly important. A preferred amplification program for amplifying template HLA gene sequences where both primers are in solution is the following 6-stage program:

1.) 1 Cycle 97 °C for 20 seconds

2.) 5 Cycles 97 °C for 35 seconds, 61 °C for 45 seconds, 72 °C for 40 seconds

3.) 25 Cycles 97 °C for 20 seconds, 59 °C for 45 seconds, 72 °C for 40 seconds

4.) 4 Cycles 97 °C for 20 seconds, 57 °C for 45 seconds, 72 °C for 90 seconds

5.) 1 Cycle 72 °C for 4 minutes

6.) 1 Cycle 30 °C for 1 second.

A number of controls can be used in the amplification methods described herein. They include, but are not limited to: 1. Omission of Primers - Control of spurious priming; 2. Known negative control - Control of specificity; 3. Known positive control - Control of sensitivity; 4. Omission of DNA Polymerase - Detection of nonspecific probe and/or enzyme/antibody sticking; 5. Use of irrelevant probes for hybridization - Control for hybridization; 6. Amplification of endogenous control DNA sequence - Detection of false negatives, control of DNA/RNA quality.

V. WASHING

After a hybridization step or after solid-phase PCR (e.g., amplification with an immobilized primer), a solid phase can be washed with a buffer to decrease non-specific binding, to wash away unbound primers, or to provide a solution that is more appropriate for subsequent detection of a detectable label, etc. Where an oligonucleotide has been immobilized or hybridized to an oligonucleotide on a solid support, the unbound oligonucleotides can be washed from a bound complex using variety of separation methods known in the art. There are many separation methods known in the art (e.g., filtering, sedimenting, centrifuging, decanting, precipitation, etc.) that can be used or adapted for use in the present invention. For example, where the amplification product is immobilized on a microtiter plate, the unbound oligonucleotides can be aspirated from the well, leaving behind those amplification products, HLA allele sequences, etc. that are

bound to a solid phase. Another separation method is the immobilization of an amplification product, HLA allele sequence, etc. on a paramagnetic bead, and the decantation or aspiration of the unbound primers and oligonucleotides leaving behind the bound complex containing a detectable label remaining on the solid phase. Commercial kits, methods and systems are commercially available and can be adapted or used with the present invention (e.g., the KingFisher™ system from Thermo Labsystems, Inc.).

A wash buffer can contain a detergent, or other agents, and compositions that are compatible with retention of the bound complex on the solid phase. A blocking agent is generally present in the wash buffer. Blocking agents include, but are not limited to non-fat dry milk, herring sperm DNA, dextran sulfate, and BSA. For example, a wash buffer that can be used in the present invention is a solution of 0.1 % BSA in PBS. The use of 0.1 % BSA results in optimum results. One or more washes may be necessary to achieve optimum lowering of non-specific binding.

VI. DETECTION

A wide range of methods can be used to detect the presence of oligonucleotides that contain a detectable label. The method of detection depends on the nature of the detectable label that is present. If the label is directly or indirectly capable of generating a signal in the visible light range, then a spectrophotometer can be used. Similarly, a fluorescent detectable label or signal generated therefrom can be measured using a fluorescent spectrophotometer. Alternatively, luminometers can be used to measure chemiluminescent signals. Isotopic labels can be measured using a liquid scintillation counter or in some cases – x-ray film. In certain embodiments, it is preferred to use a spectrophotometric plate reader that can read microtiter plates in an automated system.

VII. ANALYSIS OF RESULTS OF ASSAYS

Computer programs containing algorithm(s) can be used to score, interpret and assign HLA alleles in certain embodiments of the present invention. Briefly, the data from a detection instrument (e.g., a spectrophotometer, an ELISA reader, a scintillation counter, etc.) can be analyzed through the use of a computer program that compares the values of each sample against a reference value(s).

For example, computer programs for the ELISA format readers take readings below a designated threshold and label such as negative and values above the

same thresholds as positives. A positive well or a combination of certain wells would then represent a specific gene sequence or allele and be scored as such with the automated program. The optical density (O.D.) values obtained from reading of the wells of the ELISA plate readers are given as numerical values ranging from 0.000-2.000. This information is automatically downloaded onto the attached computers via the vendor provided software. The O.D. values are saved in a spreadsheet format in the vendor provided program as raw data.

The first step in computer analysis of the data is to validate and assign the negative control reading from the negative control well, which always exists in the same well location on the plate. A properly performed negative control is assigned as the negative value. In some embodiments where peroxidase is used with TMB, negative controls are deemed properly performed when the O.D. values are below 0.2. The usual O.D. values of a negative control reaction yielding colorless products are usually between 0.05 and 0.1. Then the threshold level is determined for that particular reaction to be 3.5 times the value of the negative control. A well is considered weakly positive if the reaction yields an O.D. reading that exceeds 3.0-fold but is below 3.5-fold of the negative control reading. A weakly positive well is rejected if two other strongly positive alleles are present for that locus. In the absence of two other strongly positive alleles for each locus, the weakly positive well is accepted if it is confirmed with repeat testing or alternative methods. A truly positive well is assigned when the O.D. readings exceed 3.5-fold over the value of the negative control. The computer program analyzes the results of all the wells, determines the positive wells based on the established criteria, and assigns the alleles based on which primer pairs exist in the positive wells. If more than two alleles are identified per locus, then the results have to be analyzed using the following protocol and confirmed by repeat testing or alternative methods.

By storing numerical reading values for the various primer pairs, many different type of assessment are possible. For example, the effects of the changes in primer pairs and primer sequences on average O.D. readings can be assessed. Consistently weak reacting sets can be replaced with primer pairs giving more robust and consistent results. Alternatively, if a particular weak reacting set of primers have no substitute, then handicap scores can be given. A more consistent tray can be developed by using the reading values as a point of reference.

VIII. HIGH THROUGHPUT METHODS AND SYSTEMS.

In the present invention, high-throughput analysis of HLA genotypes can be performed using automated devices. For example, an automated workstation (see e.g., U.S. Patent No. 5,139,744, "Automated laboratory workstation having module identification means") can be used to perform many of the steps involved in the present invention. An "automated workstation" is typically a computer-controlled apparatus which can, through robotic functions, transfer, mix, and remove liquids from microtiter plates. An automated workstation can also contain a built-in plate reader, which can read the absorbance of a liquid in a microtiter well. The automated workstation can be programmed to carry out a series of mixing, transfer, and/or removal steps. The automated workstation will typically have a multi-channel pipettor which can pipette small amounts of liquid (e.g., microliter amounts) from a vessel to the well.

For example, in some embodiments of the present invention, the automated workstation can be used to transfer DNA samples, oligonucleotides, amplification reagents. The automated work station can also be used to wash the samples using wash buffer. In addition, detection of oligonucleotides containing a detectable label can be carried out using an automated workstation. For example, the automated workstation can be used to add a detection reagent to the wells. The automated workstation, when equipped with a plate reader, can monitor the absorbance of the reaction of the detection reagent in the wells.

A number of robotic fluid transfer systems/automated work stations are available, or can easily be made from existing components. For example, a Zymate XP (Zymark Corporation; Hopkinton, MA) automated robot using a Microlab 2200 (Hamilton; Reno, NV) pipetting station can be used to transfer parallel samples to 96 well microtiter plates to set up several parallel simultaneous ligation reactions. Other automatic microplate dispensers include Lambda Jet and Lambda Dot (One Lambda, Inc. CA), and various other automatic plate washers and dispensers (*e.g.* from Thermo Labsystems, Inc. or Molecular Devices, Inc.). Moreover, it will be apparent to those of skill in the art that the PCR setup, reagent addition and washing steps can be automated with existing robotics outlined above.

Optical images viewed (and, optionally, recorded) by a camera or other recording device (*e.g.*, a photodiode and data storage device) are optionally further processed in any of the embodiments herein, *e.g.*, by digitizing the image and storing

and analyzing the image on a computer. A variety of commercially available peripheral equipment and software is available for digitizing, storing and analyzing a digitized video or digitized optical image, *e.g.*, using PC (Intel x86 or Pentium chip- compatible DOS OS2 WINDOWS, WINDOWS NT or WINDOWS 98 based machines), MACINTOSH, or UNIX based (*e.g.*, SUN work station) computers.

One conventional system carries light from the specimen field to a cooled charge-coupled device (CCD) camera, in common use in the art. A CCD camera includes an array of picture elements (pixels). The light from the specimen is imaged on the CCD. Particular pixels corresponding to regions of the specimen (*e.g.*, individual hybridization sites on an array of biological polymers) are sampled to obtain light intensity readings for each position. Multiple pixels are processed in parallel to increase speed. The apparatus and methods of the invention are easily used for viewing any sample, *e.g.*, by fluorescent or dark field microscopic techniques. The use of such automated machines, can minimize the existence of false positives, labor requirements, variabilities, human errors, human subjectivity, and human expertise requirements, and maximizes throughput, accuracy, sensitivity and specificity.

IX. HYBRIDIZATION OF CAPTURE OLIGONUCLEOTIDES TO HLA AMPLIFICATION PRODUCTS

Hybridization of Immobilized Capture Oligonucleotides to HLA Amplification Products

This method involves the use of immobilized oligonucleotides to capture HLA allele sequences contained in an amplification product. Briefly, HLA allele sequences are amplified from a template nucleic acid using HLA allele-specific forward and reverse primers. One or both of the amplification primers can contain a detectable label. Then the amplification products are denatured and hybridized to a locus-specific or allele-specific capture oligonucleotide that is already immobilized to a solid phase to form a detectable complex. The presence of the detectable label in the detectable complex is then measured using methods known to those of skill in the art (e.g., spectrophotometric means, a luminometer, etc.), which may require the addition of one or more detection reagents (e.g., an avidin-enzyme molecule with a colorimetric enzyme substrate).

The capture oligonucleotides possess sufficient nucleotide complementarity to the HLA allele sequences being amplified that they can hybridize to

them under stringent conditions. Typically, the HLA allele-specific forward and/or reverse primer will contain a detectable label (e.g., biotin, digoxigenin, EDANS, or a fluorescent moiety, etc.) so as to facilitate detection. Thus, this method allows for the amplification of many different HLA alleles which can be detected with, in the case of some HLA loci, as little as one capture oligonucleotide that is locus-specific. This is an advantage over previous methods, in which allele-specific capture oligonucleotides were used, as the detection of hundreds of alleles would require hundreds of allele-specific capture oligonucleotides (see e.g., Erlich *et al.* (1991) *Eur. J. Immunogenet.* 18(1-2): 33-55; Kawasaki *et al.* (1993) *Methods Enzymol.* 218:369-381). Thus, the present invention permits a great simplification and reduction in the number of oligonucleotides required to detect hundreds of HLA-alleles.

Hybridization of Free Capture Oligonucleotides to HLA Amplification Products and Subsequent Immobilization of the Detectable Complex.

In another embodiment of the present invention, the hybridization takes place in solution with capture oligonucleotide(s) and then the capture oligonucleotide is immobilized. This method involves the use of capture oligonucleotides that are hybridized in solution to HLA allele sequences contained in an amplification product and subsequent immobilization of the capture oligonucleotide to a solid phase. First, HLA allele sequences are amplified from a nucleic acid using HLA allele-specific forward and reverse primers. Then the amplification product are denatured and hybridized to a locus-specific or allele-specific capture oligonucleotide that is already immobilized to a solid phase. The capture oligonucleotides then hybridize and bind to the denatured single stranded PCR products at a suitable hybridization temperature and "capture" complementary sequences in the products onto the plate. If none or very little complementary sequences for the capture oligonucleotide are present after the nucleic acid amplification reaction (for example, if the allele sequence represented by the allele-specific PCR primers are not present in the sample DNA template, then no PCR product would be formed), then it is unlikely a detectable complex will form. The capture oligonucleotides possess sufficient nucleotide complementarity to the HLA allele sequences being amplified that they can hybridize to them under stringent conditions. Typically, the HLA allele-specific forward and/or reverse primer will contain a detectable

label (e.g., biotin, digoxigenin, EDANS, or a fluorescent moiety, etc.) so as to facilitate detection.

In this method, capture oligonucleotides with either conserved sequences (e.g., locus-specific oligonucleotides) or allele specific sequences can be used. The later offering an additional level of specificity whereas the former offers convenience and ease of setup as well as lower cost in having fewer sets of oligonucleotides. Thus, this method allows for the amplification of many different HLA alleles which can be detected with, in the case of some HLA loci, as little as one capture oligonucleotide that is locus-specific. This is an advantage over previous methods, in which allele-specific capture oligonucleotides were hybridized in solution to a locus-specific HLA amplification product, as the detection of hundreds of alleles would require hundreds of allele-specific capture oligonucleotides (see e.g., Nevinny-Stickel and Albert (1993) *Eur. J. of Immunogenet.*, 20: 419-427). Thus, the present invention permits a great simplification and reduction in the number of oligonucleotides required to detect hundreds of HLA-alleles.

X. AMPLIFICATION OF HLA SEQUENCES WITH IMMOBILIZED PRIMERS

This method involves the amplification of HLA sequences using allele-specific primers, where one of the pair of amplification primers is immobilized to a solid phase. The other primer constituting the primer pair contains a detectable label and is initially free in solution. This technique is not limited to the detection of HLA alleles. Essentially, any set of amplification primers and any gene can be amplified. With this method, the immobilized amplification primer serves to immobilize the amplification product directly to a solid phase. The amplification should only take place if allele that can be amplified with a particular pair of allele-specific primers is present in solution. The nucleic acid amplification and capture of PCR product take place on the same polycarbonate plate and the capture oligonucleotide/PCR primer is an allele specific sequence that identifies the sequence of interest (*e.g.* the particular HLA allele) and serves three purposes. First, it serves as the capture oligonucleotide and immobilizes the PCR products onto the plates. Second, it serves as one of the PCR primers that facilitate the nucleic acid amplification reaction. Third, it serves as the discriminating sequence that allows identification of the correct allele. This means that the PCR amplification reaction would only take place if the correct sequences that is perfectly complementary to the template (which is the particular allele of the person whose HLA sequence or other

sequence is being typed) is present on both PCR primers. An advantage of this method is the elimination of transfer, reduction of an additional set of oligonucleotides to the assay vessel (compared with two previous methods described under Section X).

If a sequence specific nucleic acid amplification reaction occurred due to perfect matching between the PCR primers and the template sequences, then the product would be immobilized on the solid phase. Following capture, the unbound non-specific labeled PCR primers can be washed off. With fluorescent probes, the plate can be read with an automated fluorescent ELISA format reader. With colorimetric reactions that are associated for example with avidin conjugated enzyme and substrate systems (e.g. avidin-conjugated horseradish peroxidase and TMB), a photometric ELISA format reader would be able to quantitate the result.

XI. MULTIPLEXING OF POSITIVE CONTROLS

In certain embodiments, one or more positive control can be added to each reaction vessel. For example, a positive control in every well can be used to distinguish from the allele specific reactions by virtue of having a different fluorophore or enzyme-substrate combinations. For example, if the allele specific reaction and the positive control use different fluorophores, then the excitation and emission wavelengths for both fluorophores can be used. The positive control amplified fragment would be longer than the allele specific reaction so that the allele specific reaction would be favored. The positive controls would be captured by the same capture probe as the allele specific if the capture probe is locus-specific. If allele-specific capture probes are used, then the positive controls may have complementary sequences to the allele specific capture probes at its 5' end of the primer that is labeled.

XII. MAGNETIC BEAD VARIATION

This method takes advantage of a commercially available nucleic acid purification method that employs magnetic beads coated with avidin or other materials to facilitate the "fishing" of the appropriate nucleic acid product of interest (KINGFISHER™ available from Thermo Labsystems, Inc.). For example, if biotinylated oligonucleotide PCR primers are used, then a biotinylated PCR product will be captured with the avidin on the beads. The magnetic beads are then pulled out of the reaction well, washed and all non-biotinylated materials will be washed off. The biotinylated products and primers are then separated from avidin coated beads by further treatment, such as

elution with excess free biotin. Thereafter, the biotinylated products are hybridized to the capture probe of interest and separated from the biotinylated primers. Alternatively, a labeled hybridization probe is allowed to bind to the PCR product, followed by washing using the KINGFISHER™ method to remove any unbound non-specific signals. Lastly, the signals would be measured. Instead of biotinylated beads, covalently modified beads that attach to PCR oligonucleotides can also be used.

XIII. SSOP WITH MOLECULAR BEACON DETECTION

In the methods of the present invention, molecular beacon oligonucleotides can be used to hybridize with allele-specific amplification products. Once the modular beacons are hybridized to a complementary sequence in an amplified product, the quencher group is no longer close enough to quench the fluorophore. As a consequence the fluorophore can be detected and quantitated. These molecular beacon oligonucleotides are known in the art and can be readily designed (see Materials section on design and construction of molecular beacon oligonucleotides). These oligonucleotides have the advantage of being directly assayable with a device that can measure fluorescence. In addition, this method can exhibit lower background signal than other methods as only oligonucleotides that are incorporated into an allele-specific product will give off a signal. Thus, molecular beacon detection does not require the addition of a detection reagent to observe whether an HLA genotype is present in an analyte.

XIV. IN SITU AMPLIFICATION VARIATION

In certain embodiments, the *in situ* amplification method is chosen to eliminate the need for DNA extraction and preparation. In contrast to the usual limitations of *in situ* amplification where the number of cycles has to be curtailed to prevent the floating away of amplified products from the cell, it is irrelevant whether amplified product stays in the cell or out. As a result, the same number of cycles can be used to generate the same degree of amplification as traditional PCR. If molecular beacon method is not incorporated into the protocol, then the reaction products from the wells will be transferred to another microtiter plate that has surface attached capture oligonucleotide probes that are similar to the ones described earlier with either conserved sequences which can be used in all the wells or allele specific sequences. By using an *in situ* amplification method it is then possible to use molecular beacons to detect the amplified products. *In situ* amplification can be carried out on a microscopic slide, a tissue sample, a microtiter plate, etc.

The molecular beacon method can be incorporated to eliminate even the washing step as well as the need for specially modified plates that can be quite expensive. It also allows for real time measurement of PCR product formation. When PCR products are formed and denatured during the various cycling steps, molecular beacons would hybridize to some of the complementary single strands, thereby fluorescing and allowing real time measurement. If real time measurement is not desired, then the molecular beacon probe can be added at the end of the reaction and only wells with amplified products that are complementary to the molecular beacon would light up. Because the unbound molecular beacon does not fluoresce, washing steps may not be necessary if the signal to noise ratio is high enough.

XV. TISSUE BLOCK SECTION VARIATION

The methods of the present invention can be carried out on paraffin embedded formaldehyde fixed sections of buffy coats, umbilical cord blood clots or blood clots placed onto glass slides with grids. The same sample can be placed onto one slide and different probes are used in an *in situ* method or many samples can be placed onto the same slide and the same probe is used for all the samples. In the latter, as many sections and slides of the samples will be cut as the number of probes plus controls. This method appears to be easier for the amplification, since there is no need to separate the different probes or reactions from one another.

EXAMPLES

EXAMPLE 1 –DETECTION OF HLA ALLELES WITH PRE-IMMOBILIZED HLA LOCUS-SPECIFIC CAPTURE OLIGONUCLEOTIDES

As a first step, experiments were carried out to determine what are the optimum conditions for immobilizing a capture oligonucleotide to a plate. In this experiment, Capture Oligonucleotide1 (5'ACCGCACCCGCTCCGTCCCATTGAAGAAAT) was modified with an amine at the 5' end with a C6 linker and a biotin group on the 3' end. For the purpose of actual HLA genotyping, the Capture Oligonucleotide will not have a biotinylated 3' end. The oligonucleotide1 was incubated on a 96 well Covalent Binding Microwell plate (Xenobind™, Xenopore, Hawthorne, NJ) according to the manufacturer's instructions.

The plate was then washed three times with phosphate-buffered-saline (PBS). ExtrAvidin® Peroxidase (SIGMA) was added and allowed to incubate on the tray. The plate was washed three times with PBS. TMB substrate (3,3',5,5' – Tetramethylbenzidine) was added to the plate, 1N HCl added and tray was read at 450 nm. The current optimum conditions for oligonucleotide binding was Capture Oligonucleotide at 100 ng/ul in PBS at pH 8.8 incubated overnight at 4°C. Alternatively, binding can occur at 37 °C for 2 hours with Capture Oligonucleotide at 100 ng/ul in PBS at pH 8.8.

Amplification of HLA alleles was carried out on DNA extracted from cord blood from three donors: Sample #8, Sample #12, and Sample #18. Purification of the DNA was carried out using either the Salt extraction with ethanol precipitation method or the Qiagen QIAamp® isolation method. The amplification was carried out using oligonucleotide primers designed to hybridize to alleles in the HLA A, B, C loci for Class I and HLA DR and DQ for Class II. The sequences and location of these primers are given in Tables 1 & 2. For Examples 1, 2, and 3, the primers listed in Tables 5 and 6 were biotinylated.

All primers are adjusted to their optimum concentration of 100 ng/ul. Primer pair mixes were set up to aliquot into PCR trays. Two different 96 well trays are set up see Tables 3 & 4. The mixes are aliquoted into labeled 1.2 ml according to the volumes given in Tables 3 & 4.

A 96 well tray dotting machine was utilized to dot the PCR Trays. The polypropylene trays are labeled with their tray identification, i.e., Class I tray and dotting number. 200 trays can be dotted with each 1.1ml Primer Mix set. The 96 well dotting machine was adjusted to a draw volume of 250 ul and a dispense volume of 5 ul. Fifty 96 well trays at a time can be dotted. Once the primers are dotted 17.0 ul of mineral oil was added to each well. The PCR tray was then covered with adhesive tape. The trays are then boxed and stored at –20 °C until use.

HLA allele sequence amplification was accomplished by adding the DNA mixture to the PCR tray and placing the tray in a thermal cycling oven. The DNA mixture contains: 40.0 ul of DNA (50-100 ng/ul), 4.0 ul Taq polymerase (5 U/ul), and 600.0 ul PCR Mix into a labeled 1.5ml tube. For Class I HLA trays, the PCR mix contains 30 mM Ammonium Chloride, 150 mM Tris-HCl pH 8.8, 4 mM $MgCl_2$, and 166 uM dNTP. For Class II HLA trays, the PCR mix contains 100 mM KCl, 20 mM Tris HCl pH 8.8, 0.2% Triton X-100, 3.4 mM $MgCl_2$, and 166 uM dNTP.

A liquid sample dispensing machine was used to add the DNA mixture to tray PCR tray. The 250 ul dispensing syringe was employed. The machine was set to add 5.0 ul to a 96 well microtiter tray. The appropriate PCR tray was placed in the machine. The DNA mixture was vortexed and then 5.0 ul of DNA mixture was dispensed into each of the 96 wells of the PCR tray. The tray was then placed in the thermal cycling oven (BioOVen, BioTherm™ Products, MD). The PCR was carried out in the cycling oven in the following 6 stage program:

1.) 1 Cycle 97 °C for 20 seconds
2.) 5 Cycles 97 °C for 35 seconds, 61 °C for 45 seconds, 72 °C for 40 seconds
3.) 25 Cycles 97 °C for 20 seconds, 59 °C for 45 seconds, 72 °C for 40 seconds
4.) 4 Cycles 97 °C for 20 seconds, 57 °C for 45 seconds, 72 °C for 90 seconds
5.) 1 Cycle 72 °C for 4 minutes
6.) 1 Cycle 30 °C for 1 second

This 6-stage program generates the optimum PCR amplification profile for this example. After amplification, PCR product was diluted. A dilution of 1:10 with PBS pH 7.4 was optimum. Therefore, 90 ul of PBS pH 7.4 was added to the PCR product. 50.0 ul of diluted PCR product was transferred from the PCR tray to the Capture plate using the 96 well dotting machine. The machine was adjusted to draw and dispense 50.0 ul.

The capture tray was then placed in the thermal cycling oven and the one stage Capture Program was run. The Capture program for this example was as follows:. 1 Cycle of 97 °C for 6 minutes, 57 °C for 12 minutes, and 30 °C for 1 second. 100 ul of hybridization solution (PBS at pH 7.4) was added to the capture tray. Also a hybridization solution of 0.9 M NaCl, 90 mM sodium citrate, 1 mM EDTA, 0.1% Ficoll, 0.3% BSA, 0.5% SDS can be used. The tray was incubated at 45 °C for 120 minutes. After the hybridization incubation the capture plate was washed. Using the plate washer, the capture plate was rinsed three times with 200 ul PBS pH 7.4 in each well.

For detection, ExtrAvidin® Peroxidase was diluted 1:2000 in 4% BSA in PBS pH 7.4, and 50.0 ul was added to each well. The Capture tray was incubated at 37 °C for 30 minutes. Then the Capture tray was washed four times with 200 ul PBS pH 7.4 in each well by the plate washer. 50.0 ul of liquid substrate (3,3',5,5' – Tetramethylbenzidine) was added to each well and incubated at 37 °C for 30 minutes. 50.0 ul of 1N HCl was added to each well to stop the reaction. The trays are read on the

Plate reader by setting the filter to 450 nm. The plate configuration was set to default a 96 well Flat bottom microtiter plate.

Data readings are stored as a spreadsheet file. Positive reactions are identified by values over threshold. Threshold was determined by numerical values that are at least 3.5 times over the value of the negative control and the average of the negative reaction values. HLA typing results are determined by the specificity corresponding to the positive reactions. The genotypes were determined as follows: Sample #8 A*0201,A*2402, B*0701, B*3501 C*0401, DRB1*0101,DRB1*1501 DRB5*0101: Sample #12, A*0201, B*1301, B*4402, C*0601 DRB1*0403, DRB1*1401 DRB3*0101,DRB4*0101; and Sample #18 A*0101,A*1101 B*0801,B*1801, C*0701, DRB1*0901, DRB1*1403, DRB3*0301, DRB4*0101.

EXAMPLE 2 SIMULTANEOUS HYBRIDIZATION OF CAPTURE OLIGONUCLEOTIDE TO DENATURED PCR PRODUCT TO CAPTURE PLATE.

For this example, a modification of the method carried out in Example 1 was performed. In this example, the amplification product is hybridized to a capture oligonucleotide(s) in solution. The capture oligonucleotide is then immobilized on a solid phase. The complexes are washed and a detection step is then performed.

The set-up of the PCR Tray was carried out as in Example 1. The PCR amplification was carried out as in Example 1 on DNA from donors #8, #12, and #18. The DNA was purified as in Example 1. After PCR amplification, diluted capture oligonucleotide was added to the wells: 5.0 ul of capture oligonucleotide at a concentration of 50 ng/ul was added to each well. The tray was placed in a thermal cycling oven and subjected to the following capture program: 1 Cycle of 97 °C for 20 seconds, 57 °C for 60 seconds, and 30 °C for 1 second. After the capture program is run, the PCR products are now hybridized with the capture oligonucleotide. The hybridized PCR products are diluted. A dilution of 1:10 with PBS at pH 7.4 was optimum. 90.0 ul of PBS at pH 7.4 was added to each well in the PCR tray. 15.0 ul of the diluted PCR product was transferred by the 96 well dotting machine into a new covalent binding plate (Xenobind™) containing 50.0 ul of PBS at pH 7.4 in each well. The plate was incubated overnight at room temperature so that the hybridized PCR product with the capture oligonucleotide with its amine linker at the 5' end can bind to the plate.

Using the plate washer, the plate was washed twice with 0.1% BSA in PBS at pH 7.4. ExtrAvidin® Peroxidase conjugate was diluted 1:2000 in 4% BSA in

PBS at pH 7.4, and 50.0 ul was added to each well. The plate was incubated at 37 °C for 30 minutes and then washed six times with 200 ul of PBS pH 7.4 in each well by the plate washer. 50.0 ul of liquid substrate (3,3',5,5' – Tetramethylbenzidine) was added to each well and incubated at 37 °C for 30 minutes. 50.0 ul of 1N HCl was added to each well to stop the reaction. The Tray reading was carried out as in Example 1. The Analysis is carried out as in Example 1.

Four basic results were observed. A "Good" result was assigned if the value for the negative control was the same as the value of a negative allele specific primer pair. Also the value of the positive control had to be higher than the value of the negative control by a factor of at least 3.5. Furthermore, the value of all positive wells had to be 3.5 times greater than the negative wells. A "Weak" result was assigned if the signal to noise ratio is above three fold but less than the 3.5 fold necessary for comfortable discrimination between positive reactions and negative reactions. Results were identified as "Too Positive" or "Background" if the value of the negative control was within acceptable limits but some of the negative wells have values equal or above that of the positive control wells. Results of "Too Positive" were observed when the Avidin conjugate concentration was too high or if insufficient washing was performed or if there was PCR DNA contamination. An "All Negative" result would be assigned if the values of the all wells were similar to the value of the negative control well. Results of "All negative" were observed when hybridization temperatures were too stringent (above 45 °C) or if the hybridization incubation times were too short (less than one hour) or if the washing conditions were too vigorous. Dilution and washing conditions are important factors to obtain the best conditions. If the hybridization product was not diluted enough, non-specific binding would result in false positives. If the washes were not exhaustive enough, false positive results would be observed.

The use of the automatic plate washer eliminated the inconsistent results and false positives that results from accidental PCR product contamination that manual handling produces. Once the washer was employed, false positive reactions and false negative reactions were greatly reduced. This observation is most likely and logically attributed to the elimination of carryover and inconsistent washing that occurs with manual washing.

In parallel with the procedure just carried out, PCR-SSP was performed using the same primer pair sets and amplification conditions. Briefly, PCR-SSP was performed with the primers sets described and the amplification products were run on

agarose gels. The bands on the gel identified the positive reactions and a typing was obtained based on the positive reactions.

The allele assignments of donors #8, #12, and #18 using the PCR-SSP method and the inventive method of this example are given below:

Summary of Typing Results

Sample #8:

PCR-SSP: A*0201,A*24XX B*07XX, B*3501 C*0401 DRB1*0101 DRB1*1501 DRB5*0101.

Inventive Method: A*0201,A*2402, B*0701, B*3501, C*0401, DRB1*0101, DRB1*1501, DRB5*0101.

Sample #12:

PCR-SSP: A*0201, B*1301, B*44XX, C*0601, DRB1*0403, DRB1*1401, DRB3*0101, DRB4*01XX.

Inventive Method: A*0201, B*1301, B*4402, C*0601, DRB1*0403, DRB1*1401, DRB3*0101, DRB4*0101.

Sample #18:

PCR-SSP: A*0101,A*1101, B*0801, B*1801, C*0701, DRB1*0901, DRB1*14XX, DRB3*03XX, DRB4*01XX.

Inventive method: A*0101, A*1101, B*0801, B*1801, C*0701, DRB1*0901, DRB1*1403, DRB3*0301, DRB4*0101.

The HLA typing from the two methods matched and was found to be in total correlation. With these samples there was 100% specificity, that is, all positive controls or expected positive samples were detected as positive reactions with readings that were at least 3.5 fold that of negative values, and all expected negative controls or samples produce negative results. 100% sensitivity was also observed with the appropriate positive readings for the positive controls or expected positive samples.

The HLA nomenclature at the allelic level is as follows. The first letter denotes the locus, i.e. HLA A and B for Class I, or DRB for Class II. The asterisk (*) denotes DNA typing. The first two numbers designates serological level or equivalent assignments. The third and fourth numbers are the allele level subtypes that are distinguished by DNA typings. The fifth and sixth numbers are usually not displayed because these designate silent mutations, i.e. DNA substitutions that do not produce

changes in protein sequence coding of the final HLA protein antigen. The seventh number, which is usually not displayed as well, denotes a null mutation, which is a mutation that silences the expression of the allele at the protein or mRNA level. There are one to two potential alleles at each locus; however, in homozygous situations where both alleles are identical, only one allele can be identified and typed. Where there is an XX after the first two numbers, it means that only one allele can be identified. This usually means that there may be homozygosity, but in a small number of cases, there may mean that there is a allele that was not detected by the entire panel of primers either because the panel cannot be all inclusive or because the allele is new and previously undiscovered.

In all instances, positive reactions observed on the PCR-SSP agarose gels corresponded to positive OD values that are at least 3.5-fold that of negative controls or negative wells on the plate reader. In this respect, it is instructive to note that because the inventive method of this example is amenable to larger sets of primer pairs, it detects several of the alleles at a higher level of resolution than the PCR-SSP method. Hence, there were several XX assignments for the third and fourth numbers in some of the alleles tested by PCR-SSP. However, the PCR-SSP method is fully capable of typing every sample to the same degree of resolution as the inventive method of this example even though is far more laborious.

EXAMPLE 3 AMPLIFICATION OF HLA SEQUENCES WITH AN IMMOBILIZED ALLELE-SPECIFIC PRIMER.

This method involves the amplification of HLA sequences using allele-specific primers, where one of the pair of amplification primers is immobilized to a solid phase. The other primer constituting a primer pair contains a detectable label and is initially free in solution. Reference DNAs were used as the template nucleic acid. The reference DNAs are from a panel of DNA that was used for the UCLA DNA Exchange Program. Primers directed to detecting class II HLA alleles were used in this example. In this example, the following immobilization primers contained an amine group followed by a C6 linker: SEQ ID NO: 189, DR06, CGTTTCTTGGAGCAGGCTAAGTG; SEQ ID NO: 190, DR07, CGTTTCTTGGAGTACTCTACGGG; SEQ ID NO: 191, DR08, ACGTTTCTTGGAGCAGGTTAAAC; SEQ ID NO: 192, DR09, CGTTTCCTGTGGCAGCCTAAGA; SEQ ID NO: 193, DR10,

CGTTTCTTGGAGTACTCTACGTC; and SEQ ID NO: 277, DRCPT1, TGGCGTGGGCGAGGCAGGGTAACTTCTTTA. The primers were immobilized to a Xenobind™ (Covalent Binding Microwell Plates), Xenopore, Hawthorne, NJ) plate according to the manufacturer's instructions. The DNA samples were isolated from reference samples known HLA allele sequences. The amplification buffer and components are the same as in Example 1 for the class II amplification. The buffer containing Taq and the proper amplification reagents were added to the microtiter wells. The other member of the primer pairs were biotinylated at their 5' ends and were as follows: SEQ ID NO:222, DR39, TGCACTGTGAAGCTCTCAC, SEQ ID NO:223, DR40, CTGCACTGTGAAGCTCTCCA. The primers were paired in separate microtiter wells as follows for sample 219 and sample 223:

Mix	Primer 1	Primer 2	Specificity
1	DR09	DR39	DR16
2	DR09	DR40	DR15
3	DR10	DR39	DR 3A,11A,13A,14A
4	DR10	DR40	DR 3B,11B,13B,14B
5	DR08	DR39	DR 4A
6	DR08	DR40	DR 4B
7	DR07	DR39	DR 8
8	DR07	DR40	DR12
9	DR06	DR39	DR53
10	Drcapt1	DR39,40	positive control
11	none	none	none
12	none	none	none

The amplification program was carried out as in Example 1. After amplification, the plate was washed twice with 0.1% BSA in PBS at pH 7.4. ExtrAvidin® Peroxidase conjugate was diluted 1:2000 in 4% BSA in PBS at pH 7.4. 50.0 ul was added to each well. The plate is incubated at 37 °C for 30 minutes and then washed six times with 200 ul of PBS pH 7.4 in each well by the plate washer. 50.0 ul of liquid substrate (3,3',5,5' – Tetramethylbenzidine) is added to each well and incubated at 37 °C for 30 minutes. 50.0 ul of 1N HCl is added to each well to stop the reaction. In parallel with the immobilized PCR method just described, PCR-SSP using the above listed primers pairs was carried out and the samples were typed by running them on agarose gels. The results of the PCR-SSP typing method and the immobilized PCR primer method carried out in this example were in complete agreement. The expected

typing of the reference DNA and the genotypes determined using PCR-SSP and immobilized PCR of this example were the same:

DNA ID	HLA Genotype of the Reference DNA	Genotype determined by PCR-SSP and the Inventive Method
219	DR1501, DR0404	DR15, DR04B
223	DR1101, DR0403	DR3,11,13,14A, DR04B

Thus, this example shows that PCR can be carried out with an immobilized primer to successfully genotype samples for their HLA allele sequences.

EXAMPLE 4- MULTIPLEXING OF POSITIVE CONTROLS INTO EVERY WELL

A positive control in every well can be used to distinguish from the allele specific reactions by virtue of having a different fluorophore or enzyme-substrate. For example, if the allele specific reaction and the positive control use different fluorophores, then the excitation and emission wavelengths for both fluorophores will be used. The positive control amplified fragment will be longer than the allele specific reaction so that the allele specific reaction would be favored. The positive controls would be captured by the same capture probe as the allele specific if the capture probe is conserved. If the allele specific capture probes are used, then the positive controls may have complementary sequences to the allele specific capture probes at its 5' end of the primer that is labeled.

In this method, positive control primers would be used. For example, SEQ ID NO:270: 5' DPA - E (PC), 5'GATCCCCCTGAGGTGACCGTG and SEQ ID NO:271: 3'DPA - F (PC), 5'CTGGGCCCGGGGGTCATGGCC are used. SEQ ID NO: 270 would be labeled with the amine linker at the 5' end and is designated 5'PC. SEQ ID NO: 271 is the 3' positive control primer and would be labeled with a fluorophore (e.g., fluorescein at the 5' end) and is designated 3'PC - (CTGGGCCCGGGGGTCATGGCC). These primers can be added to PCR mixes and used as internal controls in each well by detected their specific fluorescent signal.

EXAMPLE 5- DETECTION OF HLA SEQUENCES USING MOLECULAR BEACON PROBES

Molecular beacon probes could be used to detect allele-specific amplification products. Briefly, amplification of HLA allele sequences using HLA-specific primers is first carried out. Then molecular beacon probes that hybridize with HLA alleles sequences are hybridized to denatured amplification products. If the molecular beacon probe hybridizes then the fluorophore is no longer quenched and fluorescence would be exhibited and detected.

Fluorophore – quencher probes would be constructed from the HLA sequences given in Table 1 and 2. The loop portion of the probe would be constructed so that the sequence matched the polymorphic sequences of the HLA sequences similar to the sequences given in Tables 1 & 2. At the 5' termini there a would be 5 nucleotides of T ending with the fluorophore (e.g. 5-(2'-aminoethyl) aminonapthtalene-1-sulfonic acid (EDANS) at the 5' end. At the 3' end there would be a poly-A tail of 5 nucleotides ending with the quencher (e.g. 4-(4'-dimethylaminophenylazo)-benzoic acid (DABCYL) at the 3' end.

Following PCR amplification, the products are denatured by incubating them at 100°C for 10 minutes and then diluted in hybridization buffer. Diluted Class I products are added to the Molecular Beacon tray containing the Class I fluorophore and quencher probes. Similarly, the Class II diluted PCR product is added to the Class II Molecular Beacon tray.

To make up the tray containing the molecular beacon primers, 0.5-1.0 uM concentration of molecular beacon primers are made. The molecular beam primers would be added to wells containing allele-specific amplification products. The Molecular Beacon tray is allowed to incubate at 45-57 °C for a period of time to allow for hybridization.

When the complementary target is encountered the fluorophore is exposed and the probe can fluoresce. The tray is read by a fluorescent reader with the excitation set at 336 nm and the emission set at 490 nm. Positive reactions are identified by strong fluorescent reading and data readings are stored as a spreadsheet file. Positive reactions are identified by values over threshold. Threshold is determined by numerical values that are at least 3 times over the value of the negative control and the average of the negative reaction values.

EXAMPLE 6 IN SITU AMPLIFICATION VARIATION

Oligonucleotide primers will be used that are designed to hybridized to the polymorphic regions of HLA A, B, C loci for Class I and HLA DR and DQ for Class II. The sequences and location of these primers are given in Tables 1 & 2. The primers listed in Tables 5 and 6 are biotinylated. All primers are adjusted to their optimum concentration of 100 ng/ul. Primer pair mixes will be set up to aliquot into PCR trays. Two different 96 well trays will be set up see Tables 3 & 4. The mixes will be aliquoted into labeled 1.2 ml tubes according to the volumes given in Tables 3 & 4. A 96 well tray dotting machine is utilized to dot the PCR Trays. The polypropylene trays are labeled with their tray identification, i.e., Class I tray and dotting number. 200 trays can be dotted with each 1.1 ml Primer Mix set. The 96 well dotting machine is adjusted to a draw volume of 250 ul and a dispense volume of 5.0 ul. Fifty 96 well trays at a time can be dotted. Once the primers are dotted 17.0 ul of mineral oil is added to each well. The PCR tray is then covered with adhesive tape. The trays are then boxed and stored at – 20°C until use.

The sample would be a cell prep containing nucleated cells, or a crude cell prep with inhibitory proteins (heme) removed. First, 50-100 mg of cell prep are diluted in 100-200 ul of dH2O. Then Proteinase K (20 mg/ml) (Fisher Scientific) would be added (100 ul is used for every 50 mg of cell prep) and the sample is incubated to digest proteins in the sample. The lysate sample is incubated at 100 °C for 1 minute to inactivate the Proteinase K.

PCR amplification would be accomplished by adding the DNA mixture to the PCR tray and placing the tray in a thermal cycling oven. For DNA mixture aliquot - lysate sample, 4.0 ul Taq polymerase (5U/ul), and 600.0 ul PCR Mix into a labeled 1.5ml tube and place on ice. The PCR buffers are the sample as in Example 1: For Class I trays, PCR Mix – 30 mM Ammonium Chloride, 150 mM TRIS-HCl pH 8.8, 4 mM $MgCl_2$, and 166 uM dNTP; For Class II trays, PCR Mix – 100 mM KCl, 20 mM TRIS HCl pH 8.8, 0.2% Triton X-100, 3.4 mM $MgCl_2$, and 166 uM dNTP.

A Liquid Sample Dispensing machine would be used to add the DNA mixture to tray PCR tray. The 250 ul dispensing syringe would be employed. The machine would be set to add 5.0 ul to a 96 well microtiter tray. The appropriate PCR tray would be placed in the machine. The DNA mixture would be vortexed and then 5.0 ul of DNA mixture would be dispensed into each of the 96 wells of the PCR tray. The tray would then be placed in the thermal cycling oven.

After PCR amplification, diluted capture oligonucleotide would be added to the wells. 5.0 ul of capture oligonucleotide at a concentration of 50 ng/ul would be added to each well. The tray would be placed in the thermal cycling oven and a capture thermal cycle program run. After the capture thermal cycling, the PCR products are now hybridized with the capture oligonucleotide. The hybridized PCR products are diluted. A dilution of 1:10 with PBS at pH 7.4 is optimum. 90.0 ul of PBS at pH 7.4 is added to each well in the PCR tray. 15.0 ul of the diluted PCR product is transferred by the 96 well dotting machine into a new covalent binding plate containing 50.0 ul of PBS at pH 7.4 in each well. The plate would be incubated overnight at room temperature so that the hybridized PCR product with the capture oligonucleotide with its amine linker at the 5' end can bind to the plate. The unbound products are removed by washing. Using the plate washer, the plate is washed twice with 0.1% Tween 20 in PBS at pH 7.4. Avidin peroxidase conjugate is diluted 1:2000 in 4% BSA in PBS at pH 7.4. 50.0 ul is added to each well. The plate is incubated at 37 °C for 30 minutes.

The plate is washed six times with 200 ul of PBS pH 7.4 in each well by the plate washer. 50.0 ul of liquid substrate (3,3',5,5' – Tetramethylbenzidine) is added to each well and incubated at 37 °C for 30 minutes. 50.0 ul of 1N HCl is added to each well to stop the reaction. Trays are read on a microtiter plate reader by setting the filter to 450 nm. The data readings would be stored as a spreadsheet file and analyzed. Positive reactions are identified by values over threshold. Threshold is determined by numerical values that are at least 3.5 times over the value of the negative control and the average of the negative reaction values.

EXAMPLE 7- IN SITU AMPLIFICATION- MOLECULAR BEACON VARIATION

HLA-specific Molecular beacon probes would be constructed as in Example 5. A tray of molecular beacon probes would be spotted into microtiter plates. The template nucleic acid is contained in a cell prep containing nucleated cells or a crude cell prep with inhibitory proteins (heme) removed. First, 50-100 mg of cell prep are diluted in 100-200 ul of dH2O. Then Proteinase K (20mg/ml) (Fisher Scientific) would be added (100 ul is used for every 50 mg of cell prep) and the sample is incubated to digest proteins in the sample. The lysate sample is incubated at 100°C for 1 minute to inactivate the Proteinase K.

PCR amplification would be accomplished by adding the DNA mixture to the PCR tray and placing the tray in a thermal cycling oven. HLA locus-specific primers

are utilized to amplify HLA Class I and Class II products. For Class I primers are selected to amplify Class I exon 2 and exon 3 products. For Class II, primers are selected to amplify Class II exon 2 products. For DNA mixture aliquot - lysate sample, 4.0 ul Taq polymerase (5 U/ul), and 600.0 ul PCR Mix into a labeled 1.5 ml tube and place on ice. The PCR buffers are the sample as in Example 1. Following PCR amplification, the PCR product is denatured by incubation at 100 °C for 10 minutes and then diluted in hybridization buffer.

Diluted Class I products would be added to the Molecular Beacon tray containing the Class I fluorophore and quencher probes. Similarly, the Class II diluted PCR product would be added to the Class II Molecular Beacon tray. The Molecular Beacon tray is allowed to incubate at 45-57 °C for a period of time to allow for hybridization. When the complementary target is encountered the fluorophore is exposed and the probe can fluoresce. The tray is read by a fluorescent reader with the excitation set at 336 nm and the emission set at 490 nm. Positive reactions are identified by strong fluorescent reading and data readings are stored as a spreadsheet file. Positive reactions are identified by values over threshold. Threshold is determined by numerical values that are at least 3.5 times over the value of the negative control and the average of the negative reaction values.

EXAMPLE 8-TISSUE BLOCK SECTION VARIATION

The tissue block section method is a variation of the molecular beacon method with the use of a paraffin embedded tissue sample. The construction of the fluorophore-quencher probe is carried out as in Example 8 (Construction of the fluorophore-quencher probe). Molecular beacon tray set up would be carried out as in Example 8.

The amplification of sequences on a paraffin block sample would occur on a glass slide which will necessitate dotting the PCR mixes on a glass slide. Samples embedded in paraffin are sectioned and each slide would be added to a glass slide. The specific primer mix and DNA mixture would be added to an individual glass slide. HLA locus primers are utilized to amplify HLA Class I and Class II products. For Class I primers are selected to amplify Class I exon 2 and exon 3 products. For Class II, primers are selected to amplify Class II exon 2 products. There will be 96 individual slide made to complete the Class I or Class II sets. After adding the mix, the glass slide would be sealed with a cover slip. The slides are placed in the thermal cycling oven and the PCR

program for slides would be run. 1 cycle of 96 °C for 30 seconds followed by 34 cycles of 96 °C for 30 seconds, 61 °C for 60 seconds, 72 °C for 60 seconds.

Following PCR amplification, the PCR product would be denatured by incubation at 100 °C for 10 minutes and then diluted in hybridization buffer (0.9 M NaCl, 90 mM sodium citrate, 1 mM EDTA, 0.1% Ficoll, 0.3% BSA, and 0.5% SDS). Diluted Class I products are added to the Molecular Beacon tray containing the Class I fluorophore and quencher probes. Similarly, the Class II diluted PCR product would be added to the Class II Molecular Beacon tray. The Molecular Beacon tray would be allowed to incubate at 45-57 °C for 1 hour to allow for hybridization.

When the complementary target is encountered the fluorophore is exposed and the probe can fluoresce. The tray would be read by a fluorescent reader with the excitation set at 336 nm and the emission set at 490 nm. Positive reactions are identified by a strong fluorescent reading; positive reactions are identified by values over threshold. Threshold is determined by numerical values that are at least 3 times over the value of the negative control and the average of the negative reaction values. The data readings are then stored as a spreadsheet file. In this manner, HLA genotyping could be achieved.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification in their entirety for all purposes. Although the invention has been described with reference to preferred embodiments and examples thereof, the scope of the present invention is not limited only to those described embodiments. As will be apparent to persons skilled in the art, modifications and adaptations to the above-described invention can be made without departing from the spirit and scope of the invention, which is defined and circumscribed by the appended claims.

Table 1

Primer			SEQUENCE (5' - 3')																														MER
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
CI01	5'	HLA-C ex2 221-239	C	C	G	A	G	T	G	A	A	C	C	T	G	C	G	G	A	A	A												19
CI02	5'	HLA-C Ex2 249-268	T	A	C	T	A	C	A	A	C	C	A	G	A	G	C	G	A	G	G	A											20
CI03	5'	HLA B&C Ex2 210-228	C	A	C	A	G	A	C	T	G	A	C	C	G	A	G	T	G	A	G												19
CI04	5'	HLA-C Ex2 123-140	A	G	T	C	C	A	A	G	A	G	G	G	G	A	G	C	C	G													18
CI05	5'	HLA-A&C Ex2 5-25	C	C	A	C	T	C	C	A	T	G	A	G	G	T	A	T	T	T	C	T											20
CI06	3'	HLA-C Ex3 243-263	T	C	T	T	C	T	C	C	A	G	A	A	G	G	C	A	C	C	A	T											20
CI07	3'	HLA-C Ex3 243-263	C	A	G	G	T	C	A	G	T	G	T	G	A	T	C	T	C	C	A												19
CI08	3'	HLA-B&C Ex3 195-213	C	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	C	C	A												19
CI09	3'	HLA-C Ex4 234-251	C	A	G	C	C	C	C	T	C	G	T	G	C	T	G	C	A	T													18
CI10	3'	HLA-C Ex3 258-275	C	G	C	G	C	G	C	T	G	C	A	G	C	G	T	C	T	T													18
CI11	3'	HLA-C Ex3 195-213	C	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	C	A	G												19
CI12	3'	HLA-C Ex4 31-49	C	T	C	A	G	G	G	T	G	A	G	G	G	G	C	T	C	T													18
CI13	3'	HLA-C Ex3 134-151	T	G	A	G	C	C	G	C	C	G	T	G	T	C	C	G	C	A													18
CI14	3'	HLA-B & C Ex3 18-36	G	G	T	C	G	C	A	G	C	C	A	T	A	C	A	T	C	C	A												19
CI15	5'	HLA-B & C Ex3 59-76	C	C	G	C	G	G	G	T	A	T	G	A	C	C	A	G	T	C													18
CI16	3'	HLA-C Ex4 4-23	G	C	G	T	C	T	C	C	T	T	C	C	C	G	T	T	C	T	T												19
CI17	3'	HLA-C Ex4 4-23	A	G	C	G	T	C	T	C	C	T	T	C	C	C	A	T	T	C	T	T											20
CI18	5'	HLA-C Ex3 134-151	T	C	C	G	C	G	G	G	T	A	T	G	A	C	C	A	G	T	A												19
CI19	3'	HLA-C Ex3 25-42	G	C	C	C	C	A	G	G	T	C	G	C	A	G	C	C	A	A													18
CI20	5'	HLA-C Ex2 195-213	A	C	A	A	G	C	G	C	C	A	G	G	C	A	C	A	G	G													18
CI21	3'	HLA-ABC Ex3 216-233	G	A	G	C	C	A	C	T	C	C	A	C	G	C	A	C	T	C	T												19
CI22	3'	HLA-A & C Ex 3 196-214	C	C	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	C	T	G											20
CI23	3'	HLA-B & C Ex3 65-84	T	C	G	T	A	G	G	C	T	A	A	C	T	G	G	T	C	A													17
CI24	3'	HLA-C Ex3 131-148	C	C	G	C	C	G	T	G	T	C	C	G	C	G	G	C	A														19
CI25	5'	HLA-C Ex2 252-270	T	A	C	A	A	C	C	A	G	A	G	C	G	A	G	G	C	C	A												19
CI26	5'	HLA-C Ex2 253-270	A	C	A	A	C	C	A	G	A	G	C	G	A	G	G	C	C	G													18
CI27	5'	HLA-C Ex2 85-103	A	C	G	A	C	A	C	G	C	A	G	T	T	C	G	T	G	C	A												19
CI28	3'	HLA-C Ex2 229-246	G	C	G	C	A	G	G	T	T	C	C	G	C	A	G	G	C														17
CI29	3'	HLA-A Ex3 216-233	G	A	G	C	C	A	C	T	C	C	A	C	G	C	A	C	C	G													18
CI30	3'	HLA-ABC Ex3 216-233	G	A	G	C	C	A	C	T	C	C	A	C	G	C	A	C	G	T													18
CI31	3'	HLA-A Ex3 195-213	C	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	C	T	G												19
CI32	3'	HLA-A Ex3 48-64	C	C	G	C	G	G	A	G	G	A	A	G	C	G	C	C	A														17
CI33	5'	HLA-A Ex2 5-25	C	C	A	C	T	C	C	A	T	G	A	G	G	T	A	T	T	T	C	T	T										21
CI34	5'	HLA-A Ex2 168-186	C	C	G	G	A	G	T	A	T	T	G	G	G	A	C	C	T	G	C												19

Primer			SEQUENCE (5' - 3')	MER
CI35	3'	HLA-C Ex3 25-41	CCC CAG GTC GCA GCC AG	17
CI36	3'	HLA-B & C Ex3 169-185	CGC ACG GGC CGC CTC CA	17
CI37	5'	HLA-B Ex2 144-161	GCG CCG TGG ATA GAG CAA	18
CI38	5'	HLA-B Ex2 117-133	GCC GCG AGT CCG AGG AC	17
CI39	5'	HLA-B Ex2 181-199	ACC GGA ACA CAC AGA TCT G	19
CI40	5'	HLA-B Ex2 181-199	ACC GGG AGA CAC AGA TCT C	19
CI41	5'	HLA-A & B Ex2 170-188	GGA GTA TTG GGA CCG GAA C	19
CI42	5'	HLA-B Ex2 195-212	AAC ATG AAG GCC TCC GCG	18
CI43	5'	HLA-B Ex2 180-199	GAC CGG AAC ACA CAG ATC TT	20
CI44	3'	HLA-B Ex2 219-236	TAC CGA GAG AAC CTG CGC	18
CI45	5'	HLA-B Ex2 157-173	AGC AGG AGG GGC CGG AA	17
CI46	5'	HLA-B Ex2 51-68	GGG GAG CCC CGC TTC ATT	18
CI47	5'	HLA-B Ex2 192-210	CAG ATC TAC AAG GCC CAG G	19
CI48	5'	HLA-B Ex2 5-30	CCA TGA GGT ATT TCT ACA CCG	21
CI49	5'	HLA-B Ex2 180-199	GAC CGG AAC ACA CAG ATC TA	20
CI50	5'	HLA-B & C Ex2 221-238	CCG AGA GAG CCT GCG GAA	18
CI51	5'	HLA-A & B Ex2 220-238	ACC GAG AGA ACC TGC GGA T	19
CI52	5'	HLA-B Ex2 116-133	CGC CGC GAG TCC GAG AGA	18
CI53	5'	Control Primer PIC1	ATG ATG TTG ACC TTT CCA GGG	21
CI54	3'	Control Primer PICA	TTC TGT AAC TTT TCA TCA GTT GC	23
CI55	3'	HLA-B Ex3 195-213	CCT CCA GGT AGG CTC TGT C	19
CI56	3'	HLA-B & C Ex3 44-59	GAG GAG GCG CCC GTC G	16
CI57	3'	HLA-ABC Ex3 76-92	CTT GCC GTC GTA GGC GG	17
CI58	3'	HLA-B & C Ex3 77-95	ATC CTT GCC GTC GTA GGC T	19
CI59	3'	HLA-B Ex3 92-111	CGT TCA GGG CGA TGT AAT CT	20
CI60	3'	HLA-B Ex3 201-218	CGT GCC CTC CAG GTA GGT	18
CI61	3'	HLA-ABC Ex3 216-233	GAG CCA CTC CAC GCA CTC	18
CI62	3'	HLA-B Ex3 229-246	CCA GGT ATC TGC GGA GCG	18
CI63	3'	HLA-B Ex3 260-276	CCG CGC GCT CCA GCG TG	17
CI64	3'	HLA-B Ex3 262-279	TAC CAG CGC GCT CCA GCT	18
CI65	3'	HLA-B & C Ex3 10-29	GCC ATA CAT CCT CTG GAT GA	20
CI66	3'	HLA-B Ex3 18-36	CGT CGC AGC CAT ACA TCA C	19
CI67	3'	HLA-B Ex3 184-201	CTC TCA GCT GCT CCG CCT	18
CI68	3'	HLA-B & C Ex3 69-87	GTC GTA GGC GGA CTG GTC	18
CI69	3'	HLA-A & B Ex3 68-85	TCG TAG GCG TCC TGG TGG	18
CI70	3'	HLA-B Ex3 156-173	CTC CAA CTT GCG CTG GGA	18
CI71	3'	HLA-B Ex2 173-192	GTG TGT TCC GGT CCC AAT AT	20
CI72	3'	HLA-A & B Ex2 246-264	CGC TCT GGT TGT AGT AGC G	19
CI73	3'	HLA-B Ex4 168-187	GCC CAC TTC TGG AAG GTT CT	20

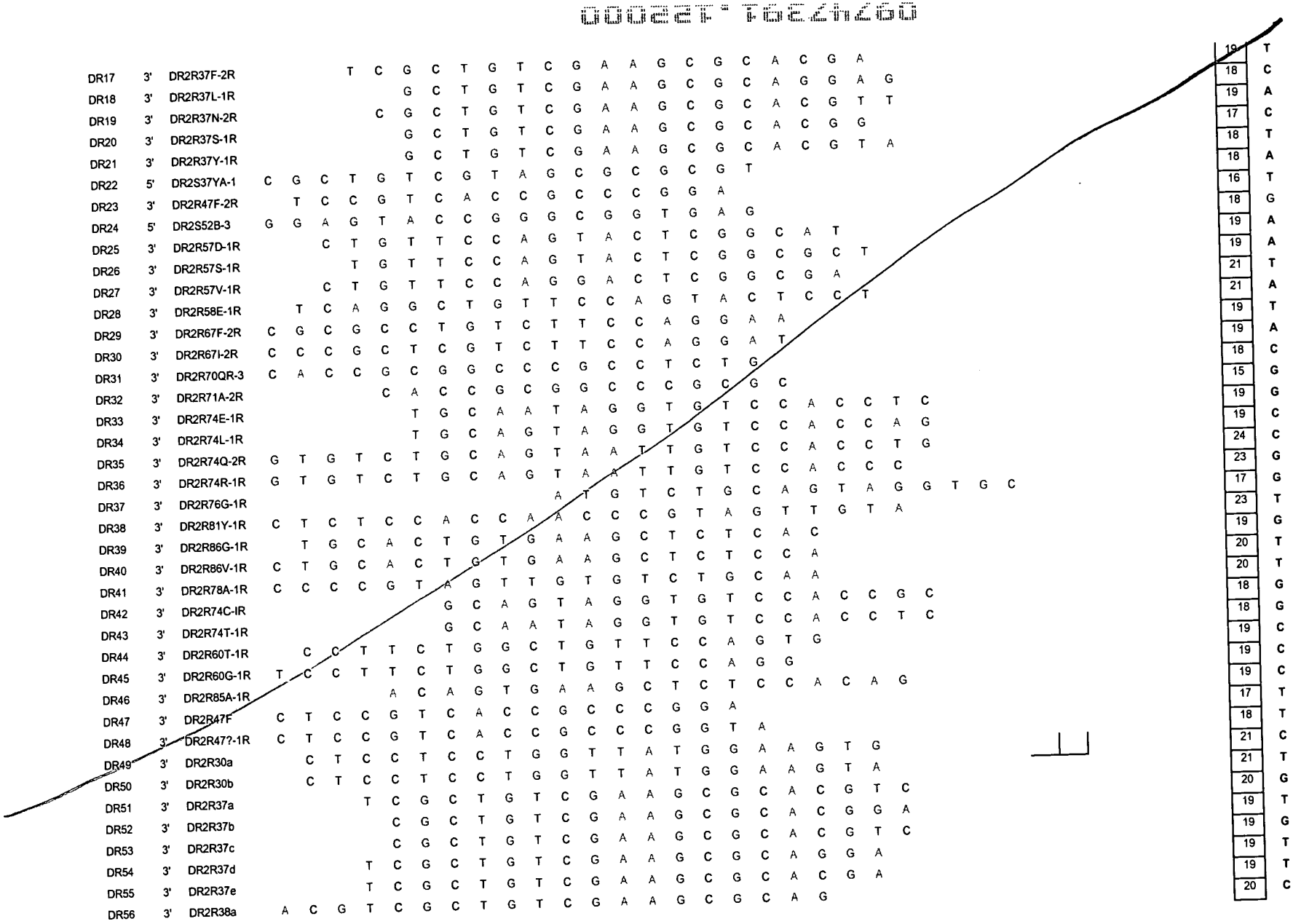
Primer			SEQUENCE (5' - 3')	MER
CI74	3'	HLA-B Ex3 11-28	C C A T A C A T C G T C T G C C A A	18
CI75	3'	HLA-B Ex2 229-245	G C G C A G G T T C C G C A G G C	17
CI76	3'	HLA-ABC Ex3 216-233	G A G C C A C T C C A C G C A C A G	18
CI77	5'	HLA-A Ex3 63-80	G G G T A C C A G C A G G A C G C T	18
CI78	5'	HLA-B & C Ex2 187-205	G A G A C A C A G A A G T A C A A G C G	20
CI79	3'	HLA-B Ex3 120-136	G C C G C G G T C C A G G A G C T	17
CI80	5'	HLA-B Ex2 222-239	C G A G A G A G C C T G C G G A A C	18
CI81	5'	HLA-B Ex2 119-136	C G C G A G T C C G A G G A T G G C	18
CI82	3'	HLA-A &B Ex3 228-245	C A G G T A T C T G C G G A G C C C	18
CI83	5'	HLA-B Ex2 5-24	C C A C T C C A T G A G G T A T T T C C	20
CI84	3'	HLA-B Ex3 120-136	G C G G C G G T C C A G G A G C G	17
CI85	3'	HLA-A & B Ex3 195-213	C C T C C A G G T A G G C T C T C A A	19
CI86	3'	HLA-B Ex2 226-243	G C A G G T T C C G C A G G C T C T	18
CI87	5'	HLA-B Ex2 244-227	G G A C C T G C G G A C C C T G C T	18
CI88	5'	HLA-B & C Ex2 52-69	G G G A G C C C C G C T T C A T C T	18
CI89	5'	HLA-B Ex2 116-133	C G C C A C G A G T C C G A G G A A	18
CI90	3'	HLA-ABC Ex3 156-172	T C C C A C T T G C G C T G G G T	17
CI91	3'	HLA-B Ex3 44-60	G G A G G A A G C G C C C G T C G	17
CI92	5'	HLA-B Ex2 227-244	G A G C C T G C G G A C C C T G C T	18
CI93	5'	HLA-B Ex2 222-239	C G A G T G G G C C T G C G G A A C	18
CI94	5'	HLA-B Ex2 76-94	G C T A C G T G G A C G A C A C G C T	19
CI95	3'	HLA-B Ex2 207-225	C T C G G T C A G T C T G T G C C T T	19
CI96	3'	HLA-B Ex2 207-226	T C T C G G T A A G T C T G T G C C T T	20
CI97	5'	HLA-A Ex2 174-192	T A T T G G G A C G A G G A G A C A G	19
CI98	3'	HLA-B & C EX3 69-87	C G T C G T A G G C G T A C T G G T C	19
CI99	5'	HLA-A Ex2 113-130	C G A C G C C G C G A G C C A G A A	18
CI100	3'	HLA-ABC Ex3 216-233	G A G C C C G T C C A C G C A C T C	18
CI101	5'	HLA-A Ex2 210-229	T C A C A G A C T G A C C G A G C G A A	20
CI102	5'	HLA-A Ex2 191-209	A C G G A A T G T G A A G G C C C A G	19
CI103	5'	HLA-A Ex2 111-127	A G C G A C G C C G C G A G C C A	17
CI104	5'	HLA-A Ex2 166-184	G G C C G G A G T A T T G G G A C G A	19
CI105	5'	HLA-A Ex2 152-170	G A T A G A G C A G G A G A G G C C T	19
CI106	5'	HLA-A & B Ex2 210-229	T C A C A G A C T G A C C G A G A G A G	20
CI107	5'	HLA-A Ex2 37-53	C C C G G C C C G G C A G T G G A	17
CI108	5'	HLA-A Ex2 149-167	G T G G A T A G A G C A G G A G G G T	19
CI109	3'	HLA-A Ex3 80-100	A T G T A A T C C T T G C C G T C G T A A	21
CI110	3'	HLA-A Ex3 212-229	C A C T C C A C G C A C G T G C C A	18
CI111	3'	HLA-A Ex3 105-123	A G C G C A G G T C C T C G T T C A A	19
CI112	3'	HLA-A Ex3 71-88	C C G T C G T A G G C G T G C T G T	18

Primer			SEQUENCE (5' - 3')	MER
CI113	3'	HLA-A Ex3 110-128	CCA AGA GCG CAG GTC CTC T	19
CI114	5'	HLA-B Ex2 189-209	ACA CAG ATC TAC AAG ACC AAC	21
CI115	5'	HLA-C Ex2 179-197	GGA CCG GGA GAC ACA GAA C	19
CI116	3'	HLA-C Ex3 25-41	CCC CAG GTC GCA GCC AC	17
CI117	3'	HLA-C EX3 183-200	TCT CAG CTG CTC CGC CGT	18
CI118	3'	HLA-C Ex3 169-186	CTC ACG GGC CGC CTC CA	17
CI119	5'	HLA-C Ex2 221-239	CCG AGT GAA CCT GCG GAA A	19
CI120	5'	HLA-C Ex2 249-268	TAC TAC AAA CCA GAG AGG GA	20
CI121	5'	HLA-B & C Ex2 210-228	CAC AGA CTG ACC GAA GAG G	19
CI122	5'	HLA-C Ex2 123-140	ATC CCA AGA GGG GAA GCC	18
CI123	5'	HLA-A & C Ex2 5-25	CCA CTC CCA TGA GTA TTT CTC	21
CI124	3'	HLA-B & C Ex3 195-213	CCT CCA GGT AGG CTC TCC A	19
CI125	3'	HLA-C Ex4 234-251	CAG CCC CTC GTG CTG CAT	18
CI126	3'	HLA-C Ex3 258-275	CGC GCG CTG CAG CGT CTT	18
CI127	3'	HLA-C Ex3 195-213	CCT CCA GGT AGG CTC TCA G	19
CI128	3'	HLA-C Ex3 18-36	GGT CGC CAG CAA ACA TCC A	19
CI129	3'	HLA-C Ex3 246-265	AGC GTC TCC TTC CCA TTC TT	20
CI130	5'	HLA-B Ex2 219-236	TAC CGA GAG AAC CTG CGC A	19
CI131	3'	HLA-B & C Ex3 76-93	CCT TGC CCG TCG TAG GCG A	18
CI132	3'	HLA-B Ex3 69-86	GTC GTA GGG CGC TCT GGT C	18
CI133	3'	HLA-A Ex3 20-39	CCA CGT CCC AGC CAT ACA ACA TT	20
CI134	5'	HLA-B & C Ex2 117-133MM	GCC GCG AGT TCG AGA GG	17
CI135	5'	HLA-B Ex2 220-238	ACC GAG AGA ACC TGT GGA T	19
CI136	3'	HLA-A Ex2 186-205	GCC TTC ACA TTC CGT GTG TT	20
CI137	3'	HLA-A Ex3 216-232	AGC CCG TCC ACG TTA TTT CAC	17
CI138	5'	HLA-A Ex2 5-25	CCA CTC CAT GAG GTA TTT CTC	21
CI139	5'	HLA-B Ex2 230-246	CCT GCG CAC CGC GTC TCC	17
CI140	3'	HLA-A & B 224-262	CTC TGG GTT GTG TAG TAG CGG GA	19
CI141	5'	HLA-A Ex3 63-80	GGG TAC CGG GCA GAC GCT	18
CI142	5'	HLA-A Ex2 191-209	ACG GAA AAG TGA AAG GCC AAG	19
CI143	?	HLA-A Ex2 184-203	CTT CAC ATT CCG GTG TCT CT	20
CI144	5'	HLA-A Ex2 89-107	CAC GCA GTT CGT GCG GTC GT	19
CI145	3'	HLA-A Ex2 226-43	GCA GGG TCC CCA GGT CCA A	18
CI146	3'	HLA-B	GCT CTG GTT GTA GTA CGC GGA	20
CI147	5'	HLA-B	GAC GAC GCG CTG GTT CGT GA	19
CI148	5'	Internal Control	TGC CAA AGT GGA GAC CCA A	19
CI149	3'	Internal Control	GCA TCT TGC TCT GTG AGG ATT	20
CI150	5'	HLA-C Ex2 5-23	ACG TCG GAG CCG TAC TGG T	18
C2F30T	5'	HLA-C Ex 2 12-30	TCC ATG AAG TAT TTC CAC ACA T	19

Primer			SEQUENCE (5' - 3')																														MER
C2F32T	5'	HLA-C Ex2 14-32	C	A	T	G	A	G	G	T	A	T	T	T	C	T	A	C	A	C	C	G	C	T									22
C2F25A	5'	HLA-C Ex2 5-25	C	A	C	T	C	C	A	T	G	A	G	G	T	A	T	T	T	C	G	A											20
C2F25C	5'	HLA-C Ex 5-25	C	A	C	T	C	C	A	T	G	A	G	G	T	A	T	T	T	C	T	C											20
C2F32C	5'	HLA-C Ex2 14-32	T	G	A	G	G	T	A	T	T	T	C	T	A	C	A	C	C	G	C	C											20
C3R195G	3'	HLA-C Ex 3 195-213	C	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	G	T	C												19
C3R195C	3'	HLA-C Ex3 195-213	C	T	C	C	A	G	G	T	A	G	G	C	T	C	T	C	C	G													18
C3R076A	3'	HLA-C Ex3 76-93	C	C	T	T	G	C	C	G	T	C	G	T	A	G	G	C	G	T													18
C3R076C	3'	HLA-C Ex3 76-93	C	C	T	T	G	C	C	G	T	C	G	T	A	G	G	C	G	G													18
C3R076T	3'	HLA-C Ex3 76-93	C	C	T	T	G	C	C	G	T	C	G	T	A	G	G	C	G	A													18
C3R075TA	3'	HLA-C Ex3 75-93	C	C	T	T	G	C	C	G	T	C	G	T	A	G	G	C	T	A													18
C2F216A	5'	HLA-C Ex2 198-216	T	A	C	A	A	G	C	G	C	C	A	G	G	C	A	C	A	G	A												19
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
CICptA1		Class I Capture Oligo A1	A	C	G	C	C	T	A	C	G	A	C	G	G	C	A	A	G	G	A	T	T	A	C	A	T	C	G	C	C	C	
CICptA2		Class I Capture Oligo A2	G	A	T	G	G	A	G	C	C	G	C	G	G	T	G	G	A	T	A	G	A	G	C	A	G	G	A	G	G	G	
CICptB1		Class I Capture Oligo B1	C	A	G	T	T	C	G	T	G	A	G	G	T	T	C	G	A	C	A	G	C	G	A	C	G	C	C				
CICptB2		Class I Capture Oligo B2	C	T	G	C	G	C	G	G	C	T	A	C	T	A	C	A	A	C	C	A	G	A	G	C	G	A	G	G	C	C	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	

Table 2

		PRIMER	SEQUENCE (5'-3')	MER	3' seq
				20	G
DQ01	5'	DQB 8V-1	T C C [CT] C G C A G A G G A T T T C G T G	15	G
DQ02	5'	DQB 26G-1	G G A G C G C G T G C G G G G	17	T
DQ03	5'	DQB 26La-1	A C G G A G C G C G T G C G T C T	19	A
DQ04	5'	DQB 26Y-2	G G A C G G A G C G C G T G C G T T A	24	C
DQ05	3'	DQB 30H-1R	G T A C T C C T C T C G G T T A T A G A T G T G	23	G
DQ06	3'	DQB 30S-1R	G A T C T C T T C T C G G T T A T A G A T G C	17	T
DQ07	3'	DQB 38V-2R	G T C G C T G T C G A A G C G C A	16	G
DQ08	5'	DQB 55P-1	T G A C G C C G C T G G G G C C	20	A
DQ09	3'	DQB 57D-2R	G C T G T T C C A G T A C T C G G C G T	21	A
DQ10	3'	DQB 57S-2R	G C T G T T C C A G T A C T C G G C G C T	20	T
DQ11	3'	DQB 57V-1R	G C T G T T C C A G T A C T C G G C A A	18	A
DQ12	3'	DQB 70R-3R	C A A C T C C G C C C G G G T C C T	21	A
DQ13	5'	DQB 71K-1	G A A G G A C A T C C T G G A G A G G A A	21	C
DQ14	3'	DQB84Q-2R	G G T C G T G C G G A G C T C C A A C T G	21	G
DQ15	3'	DQB 89G-2R	C A C T C T C C T C T G C A G G A T C C C	30	C
DQCPT1			C A C G T C G C T G T C G A A G C G C A C G T A C T C C T C	30	T
DQCPT2			C A C G T C G C T G T C G A A G C G G A C G A T C T C C T T	30	C
DQCPT3			C A C G T C G C T G T C G A A G C G T G C G T A C T C C T C	30	C
DQCPT4			C A C G T C G C T G T C G A A G C G C G C G T A C T C C T C	30	C
DQCPT5			C A C G T C G C T G T C G A A G C G C A C G T C C T C C T C	20	A
DR01	5'	DR2S9-4	C C C C [AC] C A G C A C G T T T C T T G A	19	G
DR02	5'	DR2S10G	C C A G C A C G T T T C T T G G A G G	20	T
DR03	5'	DR2S10L-1	[AC] C A G C A C G T T T C T T G G A G C T	19	A
DR04	5'	DR2S11D-2	C A C G T T T C T T G C A G C A G G A	19	G
DR05	5'	DR2S11R-1	C A C G T T T C T T G G A G C T G C G	23	G
DR06	5'	DR2S13C-2	C G T T T C T T G G A G C A G G C T A A G T G		
DR07	5'	DR2S13G-1	C G T T T C T T G G A G T A C T C T A C G G G	23	G
DR08	5'	DR2S13H-2	A C G T T T C T T G G A G C A G G T T A A A C	23	C
DR09	5'	DR2S13R-1	C G T T T C C T G T G G C A G C C T A A G A	22	A
DR10	5'	DR2S13S-2	C G T T T C T T G G A G T A C T C T A C G T C	23	C
DR11	5'	DR2S14K-2	C G T T T C C T G T G G C A G G G T A A G T A T A	25	A
DR12	3'	DR2R17-1R	G T T A T G G A A G T A T C T G T C C A G G T	23	A
DR13	5'	DR2S26L-3	C G G A G C G G G T G C G G T T G	17	G
DR14	5'	DR2S26L-4	A C G G A G C G G G T G C G G T T G	18	G
DR15	3'	DR2R30H-1R	A C T C C T C C T G G T T A T A G A A G T G	22	C
DR16	3'	DR2R37D-1R	G C T G T C G A A G C G C A A G T C	18	G


DR17 3' DR2R37F-2R
DR18 3' DR2R37L-1R
DR19 3' DR2R37N-2R
DR20 3' DR2R37S-1R
DR21 3' DR2R37Y-1R
DR22 5' DR2S37YA-1
DR23 3' DR2R47F-2R
DR24 5' DR2S52B-3
DR25 3' DR2R57D-1R
DR26 3' DR2R57S-1R
DR27 3' DR2R57V-1R
DR28 3' DR2R58E-1R
DR29 3' DR2R67F-2R
DR30 3' DR2R67I-2R
DR31 3' DR2R70QR-3
DR32 3' DR2R71A-2R
DR33 3' DR2R74E-1R
DR34 3' DR2R74L-1R
DR35 3' DR2R74Q-2R
DR36 3' DR2R74R-1R
DR37 3' DR2R76G-1R
DR38 3' DR2R81Y-1R
DR39 3' DR2R86G-1R
DR40 3' DR2R86V-1R
DR41 3' DR2R78A-1R
DR42 3' DR2R74C-IR
DR43 3' DR2R74T-1R
DR44 3' DR2R60T-1R
DR45 3' DR2R60G-1R
DR46 3' DR2R85A-1R
DR47 3' DR2R47F
DR48 3' DR2R47?-1R
DR49 3' DR2R30a
DR50 3' DR2R30b
DR51 3' DR2R37a
DR52 3' DR2R37b
DR53 3' DR2R37c
DR54 3' DR2R37d
DR55 3' DR2R37e
DR56 3' DR2R38a

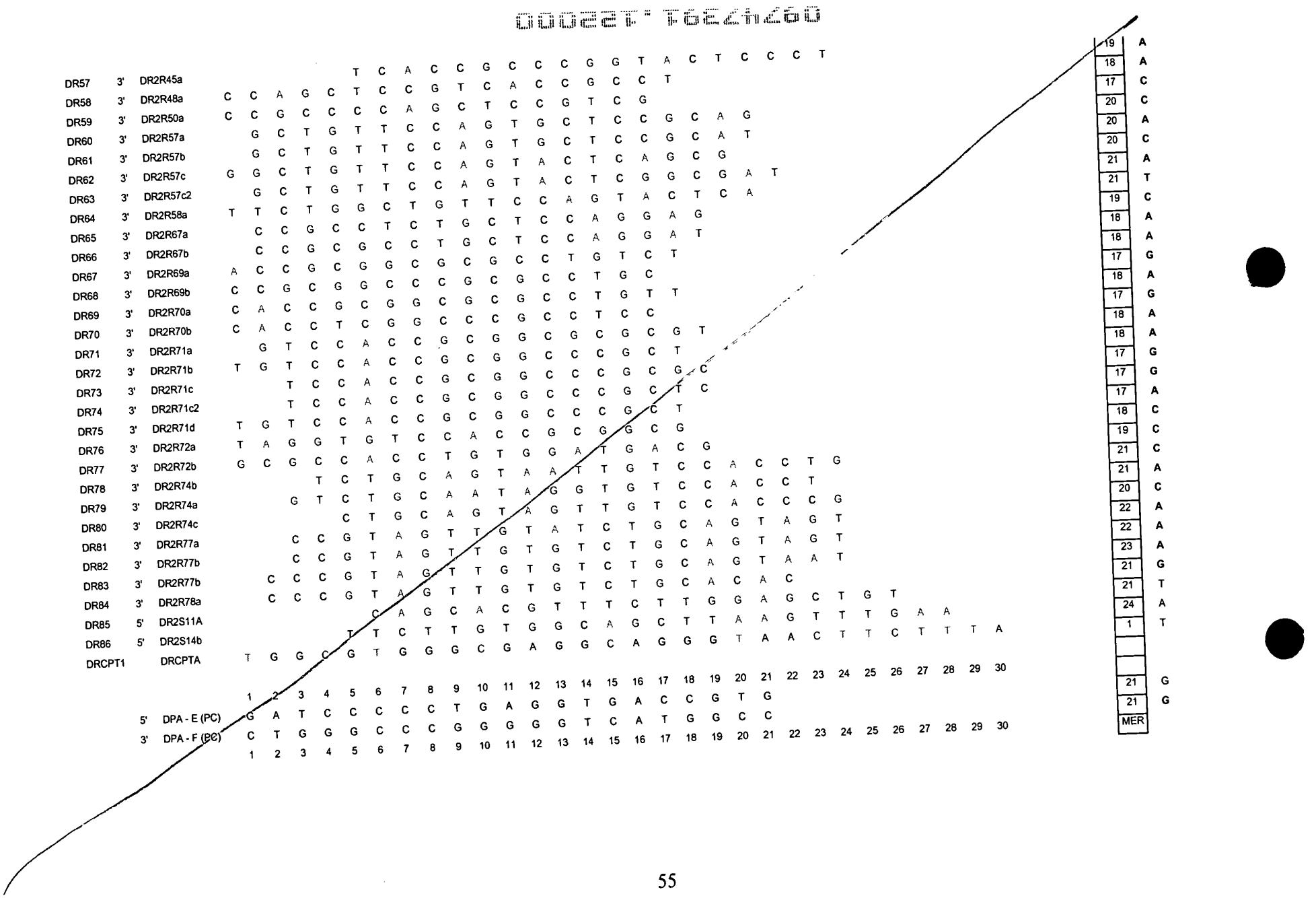

DR57 3' DR2R45a
DR58 3' DR2R48a
DR59 3' DR2R50a
DR60 3' DR2R57a
DR61 3' DR2R57b
DR62 3' DR2R57c
DR63 3' DR2R57c2
DR64 3' DR2R58a
DR65 3' DR2R67a
DR66 3' DR2R67b
DR67 3' DR2R69a
DR68 3' DR2R69b
DR69 3' DR2R70a
DR70 3' DR2R70b
DR71 3' DR2R71a
DR72 3' DR2R71b
DR73 3' DR2R71c
DR74 3' DR2R71c2
DR75 3' DR2R71d
DR76 3' DR2R72a
DR77 3' DR2R72b
DR78 3' DR2R74b
DR79 3' DR2R74a
DR80 3' DR2R74c
DR81 3' DR2R77a
DR82 3' DR2R77b
DR83 3' DR2R77b
DR84 3' DR2R78a
DR85 5' DR2S11A
DR86 5' DR2S14b
DRCPT1 DRCPTA
5' DPA - E (PC)
3' DPA - F (PC)
MER

Table 3

		5' Primer	3' Primer	Specificity	Size (bp)
A01	1	CI099	CI137	A*0101, 0102	629
A02	4	CI099	CI030	A*3601	630
A03	2	CI108	CI113	A*0201-17	489
A04	3	CI103	CI110	A*0301,0302	628
A05	15	CI102	CI029	A*1101,1102,6601	552
A06	6	CI104	CI085	A*2301	557
A07	5	CI097	CI113	A*2301,A*2401-07	464
A08	7	CI104	CI031	A*2402-05, 2407	557
A09	10	CI106	CI109	A*2501	400
A10	8	CI077	CI029,021	A*2501,2601,2603,2605,6601,6602,4301	170
A11	9	CI041	CI109	A*2501,2601-05,6601,6602,3401,3402	440
A12	11	CI101	CI109	A*2601,2602,2604,4301	400
B01	12	CI034	CI109	A*4301	442
B02	13	CI077,141	CI030	A*3401,3402	170
B03	14	CI102,142	CI109	A*3401,3402,6601,6602	419
B04	16	CI034	CI111	A*2901,2902	465
B05	17	CI107	CI112	A*3001-05	561
B06	18	CI138	CI143	A*3101	198
B07	19	CI033	CI072	A*3201	259
B08	21	CI033	CI111	A*3201,7401	628
B09	20	CI138	CI136	A*3301-03	200
B10	22	CI102	CI113	A*6801,6802,6901	447
B11	23	CI102	CI032	A*6901	383
B12	24	CI120	CI100	A*8001	494
C01	25	CI120	CI133	A*01,*11,*3601,*3401,*8001	300
C02	79	CI051	CI059	B*5101-05,51v,51GAC,5201	401
C03	80	CI041	CI059	B*5101-05,51v,51GAC,7801-02,1509	451
C04	81	CI040	CI059	B*5201	440

		5' Primer	3' Primer	Specificity	Size (bp)
C05	77	CI043	CI056,091	B*3501-09,3511,5301	389/340
C06	28	CI041	CI064	B*0702-05,8101	619
C07	29	CI114	CI064	B*0703	600
C08	30	CI043	CI055	B*0801,0802,B51GAC,B*4406	543
C09	31	CI043	CI063	B*0801,0802	606
C10	36	CI046,089	CI132,098	B*4402-06	546/481
C11	34	CI083	CI058	B*4501,45v,4901,5001	600
C12	35	CI050	CI062	B*4501,45V,1514	536
D01	42	CI081	CI058	B*1301-03	486
D02	43	CI045	CI014	B*1401,1402	389
D03	44	CI048	CI071	B*1402,3904	187
D04	67	CI081	CI086	B*1501,1502,1504-08,1511,1512,1514,1515,1519-21,1525,1526N,1528	124
D05	68	CI040	CI057	B*1501,1503-07,1512,1514,1519,1520,1524,1525,4802,4003,13x15,1526N	421
D06	70	CI052	CI057	B*1503,1518,1523,1529,4802,3907,72v,Cw0703	486
D07	72	CI039	CI076	B*1509,1510,1518,1521,1523	562
D08	73	CI081	CI062,082	B*1512,1514,1519	636/637
D09	74	CI041	CI124	B*1508,1511,1515,1522,A*68,2501,2601-05,3401,6601-02	553
D10	65	CI042	CI067	B*1516,1517	516
D11	47	CI051,139	CI060	B*3801,3802	498/508
D12	48	CI052	CI060	B*3801,3802,3901-08,6701	612
E01	45	CI050	CI060	B*3901-08,6701	507
E02	46	CI049	CI060	B*6701	548
E03	51	CI042	CI066	B*5701-03	351
E04	52	CI081	CI140	B*5701-03,1513,1516,1517,1524,1301-03,13x15	143

		5' Primer	3' Primer	Specificity	Size (bp)
E05	50	CI042	CI056	B*5801-03	374
E06	49	CI051	CI065	B*5801,5104,5301,1513	319
E07	53	CI037	CI057	B*1801,1802	458
E08	41	CI094	CI070	B*4001,4007	607
E09	40	CI089	CI061	B*4001-04,4006-08,4701	627
E10	38	CI089	CI090	B*4002-06,4008,4101,4102,4501,45v,4901,5001,4402-05,4701	566
E11	33	CI051	CI058	B*4901,5901	385
E12	32	CI094	CI067	B*4901,5001,4005,2704,2706,45v	635
F01	57	CI134	CI074	B*5401	421
F02	55	CI080	CI058	B*5401,5501,5502,5601,4501,45v,5001	383
F03	54	CI052	CI074	B*5501,5502,5601,5602,7301,3906	422
F04	56	CI047	CI076	B*5601,5602	551
F05	58	CI094	CI095,096	B*2701-09	149/150
F06	75	CI041	CI065	B*3501-04,3506-09,3511,5301,1502,1513,5104,1521,4406	369
F07	76	CI038	CI075	B*3501-13,18,7801-02,1522	128
F08	59	CI038	CI055	B*3701,B*4406,B51GAC	606
F09	60	CI040	CI131	B*3701,3902,3908	422
F10	37	CI040	CI063	B*4101,4102	605
F11	63	CI047	CI063	B*4201,42v	594
F12	66	CI078	CI079	B*4601	459
G01	61	CI040	CI069	B*4701	414

		5' Primer	3' Primer	Specificity	Size (bp)
G02	64	CI052	CI070	B*4801,8101	567
G03	39	CI040	CI084	B*4801,4001-06, weak B41	465
G04	69	CI088	CI065	B*4802	487
G05	71	CI088	CI076	B*4802,1503,1509,1510,1518,1523,1529,72v	691
G06	62	CI120	CI074	B*7301	289
G07	78	CI050	CI059	B*7801-02,1509	400
G08	26	CI051,087,092,139	CI073	Bw4	1330
G09	27	CI080	CI073	Bw6 not B73	1340
G10	82	CI121	CI116	Cw*0101,0102	341
G11	83	CI119	CI021	Cw*0201,0202,1701	522
G12	84	CI121	CI129	Cw*0302,0303,0304	565
H01	85	CI119	CI019	Cw*0401,0402	331
H02	86	CI119	CI126	Cw*0501	564
H03	87	CI120	CI014	Cw*0602	297
H04	88	CI015	CI125	Cw*0701,0702,0703	1062
H05	89	CI115	CI036	Cw*0701	516
H06	90	CI120	CI035	Cw*0702,0703	302
H07	91	CI120	CI076	Cw*0703,A*2604	494
H08	92	CI120	CI126	Cw*0704	536
H09	93	CI027	CI028,117	Cw*0802 Cw*0801/3	161/625
H10	94	CI025	CI129	Cw*0303	523

		5' Primer	3' Primer	Specificity	Size (bp)
H11	95	CI026	CI129	Cw*0302,0304	522
H12	96	Neg. Control			0

Table 4

Tray	Mix	Primer S	Primer AS	label	
A01	DRM01	DR13	DR31	DR2R70QR	DRB1*0102
A02	DRM02	DR13	DR20	DR2R37S	DRB1*0101,0102,0103,0104
A03	DRM03	DR13	DR30	DR2R67I	DRB1*0103
A04	DRM04	DR13	DR39	DR2R86G	DRB1*0101,0103
A05	DRM05	DR13	DR40	DR2R86V	DRB1*0102,0104
A06	DRM06	DR02	DR39	DR2R86G	DRB1*1001
A07	DRM07	DR02	DR25	DR2R57D	DRB1*1001
A08	DRM08	DR09	DR15	DR2R30H	DRB1*1503
A09	DRM09	DR09	DR17	DR2R37F	DRB1*1608
A10	DRM10	DR09	DR23	DR2R47F	DRB1*1501,1502,1503,1504,1505,1506,1508,1510
A11	DRM11	DR09	DR48	DR2R47?	DRB1*1507,16XX
A12	DRM12	DR09	DR25	DR2R57D	DRB1*1502
B01	DRM13	DR09	DR30	DR2R67I	DRB1*1510,1605,1607
B02	DRM14	DR09	DR29	DR2R67F	DRB1*1601,1603?,1604
B03	DRM15	DR09	DR32	DR2R71A	DRB1*15XX
B04	DRM16	DR09	DR34	DR2R74L	DRB1*1604
B05	DRM17	DR09	DR39	DR2R86G	DRB1*1502,16XX
B06	DRM18	DR09	DR40	DR2R86V	DRB1*1501,1503,1504,1505,1506,1507,1509,1510
B07	DRM19	DR10	DR12	DR17-1R	DRB1*0301,0304,5,6,8-16

Tray	Mix	Primer S	Primer AS	label	
B08	DRM20	DR10	DR21	DR2R37Y	DRB1*11XX,1303,07,11-14,17,21-25,30,33,37,38,44,45,1425
B09	DRM21	DR10	DR19	DR2R37N	DRB1*0301,02,05-15,1109,16,20,28,1301,02,05,06,09,10,15,16,18,20,26-29,31,32,34-36,39-43,1402,03,06,09,12,13,17-19,21,24,27,29,30,33
B10	DRM22	DR10	DR17	DR2R37F	DRB1*1110,12,13,17,1308,19,1401,04,05,07,08,10,11,14-16,20,22,23,26,28,31,32,34-36
B11	DRM23	DR10	DR23	DR2R47F	DRB1*0301,04,05,07-14,1101-16,18-36,38,39,1301,02,04-06,14-18,20-25,27-31,34,35?,39,41-45,1417,21,30,33,35,
B12	DRM24	DR10	DR48	DR2R47?	DRB1*0302,03,06,1117,37,1303,07,08,12,13,19,26,32,33,36-38,40,1401-16,18-20,22-29,31,32,34,36
C01	DRM25	DR10	DR25	DR2R57D	DRB1*0301-07,11,13-16,1301,02,05-11,14-20,22-25,27-29,34-37,39-42,44,1402,03,06,09,12,14,15,17-21,23,24,27,29,30,33,36
C02	DRM26	DR10	DR26	DR2R57S	DRB1*0312,1303,04,12,13,21,30,32,33,38,1413,
C03	DRM27	DR10	DR27	DR2R57V	DRB1*1331
C04	DRM28	DR10	DR28	DR2R58E	DRB1*11XX,1411,
C05	DRM29	DR10	DR29	DR2R67F	DRB1*1101,03-06,09-12,15,22-25,27-30,32,33,35,37-39,1305,07,11,14,18,21,24,26,42,1415,22,25,27
C06	DRM30	DR10	DR30	DR2R67I	DRB1*1102,14,16,20,21,1301-04,08,10,15,16,1922,23,27,28,31-41,45,1416
C07	DRM31	DR10	DR31	DR2R70QR	DRB1*1126,34,1344,1402,06,09,13,17,20,29,30,33
C08	DRM32	DR10	DR34	DR2R74L	DRB1*0820,1123,25,1313,18,1403,12,27
C09	DRM33	DR10	DR46	DR2R85?	DRB1*1106,21,1429
C10	DRM34	DR10	DR39	DR2R86G	DRB1*0302,05,09,14,17,1101,08-12,14,15,19,20,23,24,26-29,31-33,37,39,1302,03,05,07,12-14,21,23,25,26,29-31,33,34,36-39,41,45,1402,03,07,09,13,14,19,22,24,25,27,30,36
C11	DRM35	DR10	DR40	DR2R86V	DRB1*0301,03,04,06-08,10-13,15,16,0820,1102-04,06,07,13,16-18,21,25,34-36,38,1301,04,06,08-11,15,18-20,22,24,27,28,32,35,40,42-44,1401,05,06,08,12,16-18,20,21,23,26,29,32-35
C12	DRM36	DR07	DR21	DR2R37Y	DRB1*0801-08,10-15,17-19,1105,1317
D01	DRM37	DR07	DR18	DR2R37L	DRB1*1201-04,1206

Tray	Mix	Primer S	Primer AS	label	
D02	DRM38	DR07	DR23	DR2R47F	DRB1*0817,1105,1201-06,1317
D03	DRM39	DR07	DR48	DR2R47?	DRB1*0801-17,18,19,21,1404,11,15,28,31
D04	DRM40	DR07	DR26	DR2R57S	DRB1*0801,03,05,06,10,12,14,16-19w
D05	DRM41	DR07	DR25	DR2R57D	DRB1*0802,04,09,13,15,21,1105,1204,1317,1411,15
D06	DRM42	DR07	DR27	DR2R57V	DRB1*1201-03,05,06
D07	DRM43	DR07	DR28	DR2R58E	DRB1*1105,1204,1411
D08	DRM44	DR07	DR44	DR2R60?	DRB1*0808,15,1404,28,31
D09	DRM45	DR07	DR45	DR2R60?	DRB1*1201-03,05,06
D10	DRM46	DR07	DR29	DR2R67F	DRB1*0801,02,04-09,11,16,17,21,1105,1202,1415
D11	DRM47	DR07	DR34	DR2R74L	DRB1*0801-04,06-19,,21,1415
D12	DRM48	DR07	DR46	DR2R85?	DRB1*0812,1201,02,04-06,1428
E01	DRM49	DR07	DR39	DR2R86G	DRB1*0801-03,05,07-09,11,13-19,21,1105
E02	DRM50	DR07	DR40	DR2R86V	DRB1*0804,06,10,12,1201-06,1404,11,15,28,31
E03	DRM51	DR08	DR20	DR2R37S	DRB1*0406,19-21
E04	DRM52	DR08	DR21	DR2R37Y	DRB1*0401-05,07-18,22-36,1122,1410
E05	DRM53	DR08	DR23	DR2R47F	DRB1*0428,35,1122
E06	DRM54	DR08	DR26	DR2R57S	DRB1*0405,09-12,17,24,28-30
E07	DRM55	DR08	DR25	DR2R57D	DRB1*0401-04,06-08,13,14,16,18-23,25-27,31-36
E08	DRM56	DR08	DR28	DR2R58E	DRB1*0415,1122
E09	DRM57	DR08	DR29	DR2R67F	DRB1*0415,25,36,1122

Tray	Mix	Primer S	Primer AS	label	
E10	DRM58	DR08	DR30	DR2R67I	DRB1*0402,12w,14,18
E11	DRM59	DR08	DR70	DR2R70B	DRB1*0401,09,13,16,21,22,26,33-35
E12	DRM60	DR08	DR33	DR2R74E	DRB1*0403,06,07,11,17,20,22,27,1410
F01	DRM61	DR08	DR34	DR2R74L	DRB1*0412,18,25,31
F02	DRM62	DR08	DR39	DR2R86G	DRB1*0401,05,07-09,14,16,17,19-21,24,26,28-31,33-35,1122
F03	DRM63	DR08	DR40	DR2R86V	DRB1*0402-04,06,10-13,15,18,22,23,25,27,32,36,1410
F04	DRM64	DR11	DR17	DR2R37F	DRB1*0701,03,04
F05	DRM65	DR11	DR39	DR2R86G	DRB1*0701,03,04
F06	DRM66	DR01	DR27	DR2R57V	DRB1*0901
F07	DRM67	DR01	DR39	DR2R86G	DRB1*0901
F08	DRM68	DR03	DR20	DR2R37S	DRB3*0203
F09	DRM69	DR03	DR21	DR2R37Y	DRB1*1130
F10	DRM70	DR03	DR19	DR2R37N	DRB3*0206?
F11	DRM71	DR03	DR17	DR2R37F	DRB3*0301-03
F12	DRM72	DR03	DR26	DR2R57S	DRB3*0208
G01	DRM73	DR03	DR25	DR2R57D	DRB3*0107,0201-06,10-13
G02	DRM74	DR03	DR35	DR2R74Q	DRB3*0107,0201-03,05-13,0301,02
G03	DRM75	DR03	DR36	DR2R74R	DRB3*0101-06
G04	DRM76	DR03	DR39	DR2R86G	DRB3*0101-07,0202,03,05-13,0303
G05	DRM77	DR03	DR40	DR2R86V	DRB3*0201,04,0301,02

Tray	Mix	Primer S	Primer AS	label	
G06	DRM78	DR05	DR25	DR2R57D	DRB3*0107
G07	DRM79	DR05	DR39	DR2R86G	DRB3*0101-07
G08	DRM80	DR06	DR37	DR2R76G	DRB4*0102
G09	DRM81	DR06	DR38	DR2R81Y	DRB4*0101-04
G10	DRM82	DR06	DR40	DR2R86V	DRB4*0101-05
G11	DRM83	DR04	DR20	DR2R37S	NEG
G12	DRM84	DR04	DR16	DR2R37D	DRB5*0101,04-07,09
H01	DRM85	DR04	DR29	DR2R67F	DRB5*0101-05,08-10
H02	DRM86	DR04	DR32	DR2R71A	DRB5*0106,0202-04
H03	DRM87	DR04	DR34	DR2R74L	DRB5*0104
H04	DRM88	DR04	DR39	DR2R86G	DRB5*0101-05,07-10,0203
H05	DRM89	DR04	DR40	DR2R86V	DRB5*0106,0202,04,05
	Mix	P1		P2	
		15.0 ul		15.0 ul	

Table 5

ID		Location	
CI06	3'	HLA-C Ex3 243-263	Biotin
CI07	3'	HLA-C Ex3 243-263	Biotin
CI08	3'	HLA-B&C Ex3 195-213	Biotin
CI09	3'	HLA-C Ex4 234-251	Biotin
CI10	3'	HLA-C Ex3 258-275	Biotin
CI11	3'	HLA-C Ex3 195-213	Biotin
CI12	3'	HLA-C Ex4 31-49	Biotin
CI13	3'	HLA-C Ex3 134-151	Biotin
CI14	3'	HLA-B & C Ex3 18-36	Biotin
CI16	3'	HLA-C Ex4 4-23	Biotin
CI17	3'	HLA-C Ex4 4-23	Biotin
CI19	3'	HLA-C Ex3 25-42	Biotin
CI21	3'	HLA-ABC Ex3 216-233	Biotin
CI22	3'	HLA-A & C Ex 3 196-214	Biotin
CI23	3'	HLA-B & C Ex3 65-84	Biotin
CI24	3'	HLA-C Ex3 131-148	Biotin
CI28	3'	HLA-C Ex2 229-246	Biotin
CI29	3'	HLA-A Ex3 216-233	Biotin
CI30	3'	HLA-ABC Ex3 216-233	Biotin
CI31	3'	HLA-A Ex3 195-213	Biotin
CI32	3'	HLA-A Ex3 48-64	Biotin
CI35	3'	HLA-C Ex3 25-41	Biotin
CI36	3'	HLA-B & C Ex3 169-185	Biotin
CI44	3'	HLA-B Ex2 219-236	Biotin
CI55	3'	HLA-B Ex3 195-213	Biotin
CI56	3'	HLA-B & C Ex3 44-59	Biotin
CI57	3'	HLA-ABC Ex3 76-92	Biotin
CI58	3'	HLA-B & C Ex3 77-95	Biotin
CI59	3'	HLA-B Ex3 92-111	Biotin
CI60	3'	HLA-B Ex3 201-218	Biotin
CI61	3'	HLA-ABC Ex3 216-233	Biotin
CI62	3'	HLA-B Ex3 229-246	Biotin
CI63	3'	HLA-B Ex3 260-276	Biotin
CI64	3'	HLA-B Ex3 262-279	Biotin
CI65	3'	HLA-B & C Ex3 10-29	Biotin

CI66	3'	HLA-B Ex3 18-36	Biotin
CI67	3'	HLA-B Ex3 184-201	Biotin
CI68	3'	HLA-B & C Ex3 69-87	Biotin
CI69	3'	HLA-A & B Ex3 68-85	Biotin
CI70	3'	HLA-B Ex3 156-173	Biotin
CI71	3'	HLA-B Ex2 173-192	Biotin
CI72	3'	HLA-A & B Ex2 246-264	Biotin
CI73	3'	HLA-B Ex4 168-187	Biotin
CI74	3'	HLA-B Ex3 11-28	Biotin
CI75	3'	HLA-B Ex2 229-245	Biotin
CI76	3'	HLA-ABC Ex3 216-233	Biotin
CI79	3'	HLA-B Ex3 120-136	Biotin
CI82	3'	HLA-A &B Ex3 228-245	Biotin
CI84	3'	HLA-B Ex3 120-136	Biotin
CI86	3'	HLA-B Ex2 226-243	Biotin
CI90	3'	HLA-ABC Ex3 156-172	Biotin
CI91	3'	HLA-B Ex3 44-60	Biotin
CI95	3'	HLA-B Ex2 207-225	Biotin
CI96	3'	HLA-B Ex2 207-226	Biotin
CI98	3'	HLA-B & C EX3 69-87	Biotin
CI100	3'	HLA-ABC Ex3 216-233	Biotin
CI109	3'	HLA-A Ex3 80-100	Biotin
CI110	3'	HLA-A Ex3 212-229	Biotin
CI111	3'	HLA-A Ex3 105-123	Biotin
CI112	3'	HLA-A Ex3 71-88	Biotin
CI113	3'	HLA-A Ex3 110-128	Biotin
CI116	3'	HLA-C Ex3 25-41	Biotin
CI117	3'	HLA-C EX3 183-200	Biotin
CI118	3'	HLA-C Ex3 169-186	Biotin
CI124	3'	HLA-B & C Ex3 195-213	Biotin
CI125	3'	HLA-C Ex4 234-251	Biotin
CI126	3'	HLA-C Ex3 258-275	Biotin
CI127	3'	HLA-C Ex3 195-213	Biotin
CI128	3'	HLA-C Ex3 18-36	Biotin
CI129	3'	HLA-C Ex3 246-265	Biotin
CI131	3'	HLA-B & C Ex3 76-93	Biotin
CI132	3'	HLA-B Ex3 69-86	Biotin
CI133	3'	HLA-A Ex3 20-39	Biotin

CI136	3'	HLA-A Ex2 186-205	Biotin
CI137	3'	HLA-A Ex3 216-232	Biotin
CI140	3'	HLA-A & B 224-262	Biotin
CI143	3'	HLA-A Ex2 184-203	Biotin
CI145	3'	HLA-A Ex2 226-43	Biotin
CI146	3'	HLA-B	Biotin
CI149	3'	Internal Control	Biotin
C3R195G	3'	HLA-C Ex 3 195-213	Biotin
C3R195C	3'	HLA-C Ex3 195-213	Biotin
C3R076A	3'	HLA-C Ex3 76-93	Biotin
C3R076C	3'	HLA-C Ex3 76-93	Biotin
C3R076T	3'	HLA-C Ex3 76-93	Biotin
C3R075TA	3'	HLA-C Ex3 75-93	Biotin

Table 6

ID			PRIMER
DQ01	5'	Biotin	DQB 8V-1
DQ02	5'	Biotin	DQB 26G-1
DQ03	5'	Biotin	DQB 26La-1
DQ04	5'	Biotin	DQB 26Y-2
DQ08	5'	Biotin	DQB 55P-1
DQ13	5'	Biotin	DQB 71K-1
DR01	5'	Biotin	DR2S9-4
DR02	5'	Biotin	DR2S10G
DR03	5'	Biotin	DR2S10L-1
DR04	5'	Biotin	DR2S11D-2
DR05	5'	Biotin	DR2S11R-1
DR06	5'	Biotin	DR2S13C-2
DR07	5'	Biotin	DR2S13G-1
DR08	5'	Biotin	DR2S13H-2
DR09	5'	Biotin	DR2S13R-1
DR10	5'	Biotin	DR2S13S-2
DR11	5'	Biotin	DR2S14K-2
DR12	3'		DR2R17-1R
DR13	5'	Biotin	DR2S26L-3
DR14	5'	Biotin	DR2S26L-4
DR22	5'	Biotin	DR2S37YA-1
DR24	5'	Biotin	DR2S52B-3
DR85	5'	Biotin	DR2S11A
DR86	5'	Biotin	DR2S14b
	5'	Biotin	DPA - E (PC)

SEQUENCE LISTING

1. SEQ ID NO:1:C101	CCGAGTGAACCTGCGGAAA
2. SEQ ID NO:2:C102	TACTACAACCAGAGCGAGGA
3. SEQ ID NO:3:C103	CACAGACTGACCGAGTGAG
4. SEQ ID NO:4:C104	AGTCCAAGAGGGGAGCCG
5. SEQ ID NO:5:C105	CCACTCCATGAGGTATTTCT
6. SEQ ID NO:6:C106	TCTTCTCCAGAAGGCACCAT
7. SEQ ID NO:7:C107	CAGGTCAGTGTGATCTCCA
8. SEQ ID NO:8:C108	CCTCCAGGTAGGCTCTCCA
9. SEQ ID NO:9:C109	CAGCCCCTCGTGCTGCAT
10. SEQ ID NO:10:C110	CGCGCGCTGCAGCGTCTT
11. SEQ ID NO:11:C111	CCTCCAGGTAGGCTCTCAG
12. SEQ ID NO:12:C112	CTCAGGGTGAGGGGCTCT
13. SEQ ID NO:13:C113	TGAGCCGCCGTGTCCGCA
14. SEQ ID NO:14:C114	GGTCGCAGCCATACATCCA
15. SEQ ID NO:15:C115	CCGCGGGTATGACCAGTC
16. SEQ ID NO:16:C116	GCGTCTCCTTCCCGTTCTT
17. SEQ ID NO:17:C117	AGCGTCTCCTTCCCATTCTT
18. SEQ ID NO:18:C118	TCCGCGGGTATG ACCAGTA
19. SEQ ID NO:19:C119	GCCCCAGGTCGCAGCCAA
20. SEQ ID NO:20:C120	ACAAGCGCCAGGCACAGG
21. SEQ ID NO:21:C121	GAGCCACTCCACGCACTC
22. SEQ ID NO:22:C122	CCCTCCAGGTAGGCTCTCT
23. SEQ ID NO:23:C123	TCGTAGGCTAACTGGTCATG
24. SEQ ID NO:24:C124	CCGCCGTGTCCGCGGCA
25. SEQ ID NO:25:C125	TACAACCAGAGCGAGGCCA
26. SEQ ID NO:26:C126	ACAACCAGAGCGAGGCCG
27. SEQ ID NO:27:C127	ACGACACGCAGTTCGTGCA
28. SEQ ID NO:28:C128	GCGCAGGTTCCGCAGGC
29. SEQ ID NO:29:C129	GAGCCACTCCACGCACCG
30. SEQ ID NO:30:C130	GAGCCACTCCACGCACGT
31. SEQ ID NO:31:C131	CCTCCAGGTAGGCTCTCTG

32. SEQ ID NO:32:C132 CCGCGGAGGAAGCGCCA
33. SEQ ID NO:33:C133 CCACTCCATGAGGTATTTCTT
34. SEQ ID NO:34:C134 CCGGAGTATTGGGACCTGC
35. SEQ ID NO:35:C135 CCCCAGGTCGCAAGCCAG
36. SEQ ID NO:36:C136 CGCACGGGCCGCCTCCA
37. SEQ ID NO:37:C137 GCGCCGTGGATAGAGCAA
38. SEQ ID NO:38:C138 GCCGCGAGTCCGAGGAC
39. SEQ ID NO:39:C139 ACCGGAACACACAGATCTG
40. SEQ ID NO:40:C140 ACCGGGAGACACAGATCTC
41. SEQ ID NO:41:C141 GGAGTATTGGGACCGGAAC
42. SEQ ID NO:42:C142 AACATGAAGGCCTCCGCG
43. SEQ ID NO:43:C143 GACCGGAACACACAGATCTT
44. SEQ ID NO:44:C144 TACCGAGAGAACCTGCGC
45. SEQ ID NO:45:C145 AGCAGGAGGGGCCGGAA
46. SEQ ID NO:46:C146 GGGGAGCCCCGCTTCATT
47. SEQ ID NO:47:C147 CAGATCTACAAGGCCCAGG
48. SEQ ID NO:48:C148 CCATGAGGTATTTCTACACCG
49. SEQ ID NO:49:C149 GACCGGAACACACAGATCTA
50. SEQ ID NO:50:C150 CCGAGAGAGCCTGCGGGAA
51. SEQ ID NO:51:C151 ACCGAGAGAACCTGCGGAT
52. SEQ ID NO:52:C152 CGCCGCGAGTCCGAGAGA
53. SEQ ID NO:55:C155 CCTCCAGGTAGGCTCTGTC
54. SEQ ID NO:56:C156 GAGGAGGCGCCCGTCG
55. SEQ ID NO:57:C157 CTTGCCGTCGTAGGCGG
56. SEQ ID NO:58:C158 ATCCTTGCCGTCGTAGGCT
57. SEQ ID NO:59:C159 CGTTCAGGGCGATGTAATCT
58. SEQ ID NO:60:C160 CGTGCCCTCCAGGTAGGT
59. SEQ ID NO:61:C161 GAGCCACTCCACGCACTC
60. SEQ ID NO:62:C162 CCAGGTATCTGCGGAGCG
61. SEQ ID NO:63:C163 CCGCGCGCTCCAGCGTG
62. SEQ ID NO:64:C164 TACCAGCGCGCTCCAGCT
63. SEQ ID NO:65:C165 GCCATACATCCTCTGGATGA
64. SEQ ID NO:66:C166 CGTCGCAGCCATACATCAC
65. SEQ ID NO:67:C167 CTCTCAGCTGCTCCGCCT

66. SEQ ID NO:68:C168 GTCGTAGGCGGACTGGTC
67. SEQ ID NO:69:C169 TCGTAGGCGTCCTGGTGG
68. SEQ ID NO:70:C170 CTCCAACTTGCGCTGGGA
69. SEQ ID NO:71:C171 GTGTGTTCCGGTCCCAATAT
70. SEQ ID NO:72:C172 CGCTCTGGTTGTAGTAGCG
71. SEQ ID NO:73:C173 GCCCACTTCTGGAAGGTTCT
72. SEQ ID NO:74:C174 CCATACATCGTCTGCCAA
73. SEQ ID NO:75:C175 GCGCAGGTTCCGCAGGC
74. SEQ ID NO:76:C176 GAGCCACTCCACGCACAG
75. SEQ ID NO:77:C177 GGGTACCCAGCAGGACGCT
76. SEQ ID NO:78:C178 GAGACACAGAAGTACAAGCG
77. SEQ ID NO:79:C179 GCCGCGGTCCAGGAGCT
78. SEQ ID NO:80:C180 CGAGAGAGCCTGCGGAAC
79. SEQ ID NO:81:C181 CGCGAGTCCGAGGATGGC
80. SEQ ID NO:82:C182 CAGGTATCTGCGGAGCCC
81. SEQ ID NO:83:C183 CCACTCCCATGAGGTATTTCC
82. SEQ ID NO:84:C184 GCGGCGGTCCAGGAGCG
83. SEQ ID NO:85:C185 CCTCCAGGTAGGCTCTCAA
84. SEQ ID NO:86:C186 GCAGGTTCCGCAGGCTCT
85. SEQ ID NO:87:C187 GGACCTGCGGACCCTGCT
86. SEQ ID NO:88:C188 GGGAGCCCCGCTTCATCT
87. SEQ ID NO:89:C189 CGCCACGAGTCCGAGGAA
88. SEQ ID NO:90:C190 TCCCACTTGCGCTGGGT
89. SEQ ID NO:91:C191 GGAGGAAGCGCCCGTCG
90. SEQ ID NO:92:C192 GAGCCTGCGGACCCTGCT
91. SEQ ID NO:93:C193 CGAGTGGGCCTGCGGAAC
92. SEQ ID NO:94:C194 GCTACGTGGACGACACGGCT
93. SEQ ID NO:95:C195 CTCGGTCAGTCTGTGCCTT
94. SEQ ID NO:96:C196 TCTCGGTAAGTCTGTGCCTT
95. SEQ ID NO:97:C197 TATTGGGACGAGGAGACAG
96. SEQ ID NO:98:C198 CGTCGTAGGCGTACTGGTC
97. SEQ ID NO:99:C199 CGACGCCGCGAGCCAGAA
98. SEQ ID NO:100:CI100 GAGCCCGTCCACGCACTC
99. SEQ ID NO:101:CI101 TCACAGACTGACCGAGCGAA

100. SEQ ID NO:102:CI102 ACGGAATGTGAAGGCCCAG
101. SEQ ID NO:103:CI103 AGCGACGCCGCGAGCCA
102. SEQ ID NO:104:CI104 GGCCGGAGTATTGGGACGA
103. SEQ ID NO:105:CI105 GATAGAGCAGGAGAGGCCT
104. SEQ ID NO:106:CI106 TCACAGACTGACCGAGAGAG
105. SEQ ID NO:107:CI107 CCCGGCCCGGCAGTGGA
106. SEQ ID NO:108:CI108 GTGGATAGAGCAGGAGGGT
107. SEQ ID NO:109:CI109 AGTTAATCCTTGCCGTCGTAA
108. SEQ ID NO:110:CI110 CACTCCACGCACGTGCCA
109. SEQ ID NO:111:CI111 AGCGCAGGTCCTCGTTCAA
110. SEQ ID NO:112:CI112 CCGTCGTAGGCGTGCTGT
111. SEQ ID NO:113:CI113 CCAAGAGCGCAGGTCCTCT
112. SEQ ID NO:114:CI114 ACACAGATCTACAAGACCAAC
113. SEQ ID NO:115:CI115 GGACCCGGGAGACACAGAAC
114. SEQ ID NO:116:CI116 CCCCAGGTCGCAGCCAC
115. SEQ ID NO:117:CI117 TCTCAGCTGCTCCGCCGT
116. SEQ ID NO:118:CI118 CTCACGGGCCGCCTCCA
117. SEQ ID NO:119:CI119 CCGAGTGAACCTGCGGAAA
118. SEQ ID NO:120:CI120 TACTACAACCAGAGCGAGGA
119. SEQ ID NO:121:CI121 CACGACTGACCGAGTGAG
120. SEQ ID NO:122:CI122 AGTCCAAGAGGGGAGCCG
121. SEQ ID NO:123:CI123 CCACTCCATGAGGTATTTCTC
122. SEQ ID NO:124:CI124 CCTCCAGGTAGGCTCTCCA
123. SEQ ID NO:125:CI125 CAGCCCCTCGTGCTGCAT
124. SEQ ID NO:126:CI126 CGCGCGCTGCAGCGTCTT
125. SEQ ID NO:127:CI127 CCTCCAGGTAGGCTTCAG
126. SEQ ID NO:128:CI128 GGTCGCAGCCAAACATCCA
127. SEQ ID NO:129:CI129 AGCGTCTCCTTCCCATTCTT
128. SEQ ID NO:130:CI130 TACCGAGAGAACCTGCGCA
129. SEQ ID NO:131:CI131 CCTTGCCGTCGTAGGCGA
130. SEQ ID NO:132:CI132 GTCGTAGGCGTCCTGGTC
131. SEQ ID NO:133:CI133 CCACGTCGCAGCCATACATT
132. SEQ ID NO:134:C134 GCCGCGAGTTCGAGAGG
133. SEQ ID NO:135:CI135 ACCGAGAGAACCTGCGGAT

134. SEQ ID NO:136:CI136 GCCTTCACATTCCGTGTGTT

135. SEQ ID NO:137:CI137 AGCCCGTCCACGCACCG

136. SEQ ID NO:138:CI138 CCACTCCATGAGGTATTTCAC

137. SEQ ID NO:139:CI139 CCTGCGCACCGCGCTCC

138. SEQ ID NO:140:CI140 CTCTGGTTGTAGTAGCGGA

139. SEQ ID NO:141:CI141 GGGTACCGGCAGGACGCT

140. SEQ ID NO:142:CI142 ACGGAAAGTGAAGGCCCAG

141. SEQ ID NO:143:CI143 CTTCACATTCCGTGTCTCCT

142. SEQ ID NO:144:CI144 CACGCAGTTCGTGCGGTTT

143. SEQ ID NO:145:CI145 GCAGGGTCCCCAGGTCCA

144. SEQ ID NO:146:CI146 GCTCTGGTTGTAGTAGCGGA

145. SEQ ID NO:147:CI147 GACGACACGCTGTTCGTGA

146. SEQ ID NO:148:CI150 ACGTCGCAGCCGTACATG

147. SEQ ID NO:149:C2F30T TCCATGAAGTATTTCACAT

148. SEQ ID NO:150:C2F32T CATGAGGTATTTCTACACCGCT

149. SEQ ID NO:151:C2F25A CACTCCATGAGGTATTTCGA

150. SEQ ID NO:152:C2F25C CACTCCATGAGGTATTTCTC

151. SEQ ID NO:153:C2F32C TGAGGTATTTCTACACCGCC

152. SEQ ID NO:154:C3R195G CCTCCAGGTAGGCTCTGTC

153. SEQ ID NO:155:C3R195C CTCCAGGTAGGCTCTCCG

154. SEQ ID NO:156:C34076A CCTTGCCGTCGTAGGCGT

155. SEQ ID NO:157:C34076C CCTTGCCGTCGTAGGCGG

156. SEQ ID NO:158:C3R076T CCTTGCCGTCGTAGGCGA

157. SEQ ID NO:159:C3R075TA CCTTGCCGTCGTAGGCTA

158. SEQ ID NO:160:C2F216A TACAAGCGCCAGGCACAGA

159. SEQ ID NO:161:CI53 ATGATGTTGACCTTTCCAGGG

160. SEQ ID NO:162:CI54 TTCTGTAACTTTTCATCAGTTGC

161. SEQ ID NO:163:CI148 TGCCAAGTGGAGCACCCAA

162. SEQ ID NO:164:CI149 GCATCTTGCTCTGTGCAGA

163. SEQ ID NO:165:CICptA1 ACGCCTACGACGGCAAGGATTACATCGCCC

164. SEQ ID NO:166:CICptA2 GATGGAGCCGCGGTGGATAGAGCAAGGAGGG

165. SEQ ID NO:167:CICptB1 CAGTTCGTGAGGTTCGACAGCGACGCC

166. SEQ ID NO:168:CICptB2 CTGCGCGGCTACTACAACCAGAGCGAGGCC

167. SEQ ID NO:169:DQ01 TCC[CT]CGCAGAGGATTTCGTG

168. SEQ ID NO:170:DQ02 GGAGCGCGTGCGGGG

169. SEQ ID NO:171:DQ03 ACGGAGCGCGTGCGTCT

170. SEQ ID NO:172:DQ04 GGACGGAGCGCGTGCGTTA

171. SEQ ID NO:173:DQ05 GTACTCCTCTCGGTTATAGATGTG

172. SEQ ID NO:174:DQ06 GATCTCTTCTCGGTTATAGATGC

173. SEQ ID NO:175:DQ07 GTCGCTGTCGAAGCGCA

174. SEQ ID NO:176:DQ08 TGACGCCGCTGGGGCC

175. SEQ ID NO:177:DQ09 GCTGTTCCAGTACTCGGCGT

176. SEQ ID NO:178:DQ10 GCTGTTCCAGTACTCGGCGCT

177. SEQ ID NO:179:DQ11 GCTGTTCCAGTACTCGGCAA

178. SEQ ID NO:180:DQ12 CAACTCCGCCCGGGTCCT

179. SEQ ID NO:181:DQ13 GAAGGACATCCTGGAGAGGAA

180. SEQ ID NO:182:DQ14 GGTCGTGCGGAGCTCCAACTG

181. SEQ ID NO:183:DQ15 CACTCTCCTCTGCAGGATCCC

182. SEQ ID NO:184:DR01 CCCC[AC]CAGCACGTTTCTTGA

183. SEQ ID NO:185:DR02 CCAGCACGTTTCTTGGAGG

184. SEQ ID NO:186:DR03 [AC]CAGCACGTTTCTTGGAGCT

185. SEQ ID NO:187:DR04 CACGTTTCTTGCAGCAGGA

186. SEQ ID NO:188:DR05 CACGTTTCTTGGAGCTGCG

187. SEQ ID NO:189:DR06 CGTTTCTTGGAGCAGGCTAAGTG

188. SEQ ID NO:190:DR07 CGTTTCTTGGAGTACTCTACGGG

189. SEQ ID NO:191:DR08 ACGTTTCTTGGAGCAGGTTAAAC

190. SEQ ID NO:192:DR09 CGTTTCCTGTGGCAGCCTAAGA

191. SEQ ID NO:193:DR10 CGTTTCTTGGAGTACTCTACGTC

192. SEQ ID NO:194:DR11 CGTTTCCTGTGGCAGGGTAAGTATA

193. SEQ ID NO:195:DR12 GTTATGGAAGTATCTGTCCAGGT

194. SEQ ID NO:196:DR13 CGGAGCGGGTGCGGTTG

195. SEQ ID NO:197:DR14 ACGGAGCGGGTGCGGTTG

196. SEQ ID NO:198:DR15 ACTCCTCCTGGTTATAGAAGTG

197. SEQ ID NO:199:DR16 GCTGTCGAAGCGCAAGTC

198. SEQ ID NO:200:DR17 TCGCTGTCGAAGCGCACGA

199. SEQ ID NO:201:DR18 GCTGTCGAAGCGCAGGAG

200. SEQ ID NO:202:DR19 CGCTGTCGAAGCGCACGTT

201. SEQ ID NO:203:DR20 GCTGTCGAAGCGCACGG

202. SEQ ID NO:204:DR21 GCTGTCGAAGCGCACGTA

203. SEQ ID NO:205:DR22 CGCTGTCGTAGCGCGCGT

204. SEQ ID NO:206:DR23 TCCGTCACCGCCCGGA

205. SEQ ID NO:207:DR24 GGAGTACCGGGCGGTGAG

206. SEQ ID NO:208:DR25 CTGTTCCAGTACTCGGCAT

207. SEQ ID NO:209:DR26 TGTTCCAGTACTCGGCGCT

208. SEQ ID NO:210:DR27 CTGTTCCAGGACTCGGCGA

209. SEQ ID NO:211:DR28 TCAGGCTGTTCCAGTACTCCT

210. SEQ ID NO:212:DR29 CGCGCCTGTCTTCCAGGAA

211. SEQ ID NO:213:DR30 CCCGCTCGTCTTCCAGGAT

212. SEQ ID NO:214:DR31 CACCGCGGCCCGCCTCTG

213. SEQ ID NO:215:DR32 CACCGCGGCCCGCGC

214. SEQ ID NO:216:DR33 TGCAATAGGTGTCCACCTC

215. SEQ ID NO:217:DR34 TGCAGTAGGTGTCCACCAG

216. SEQ ID NO:218:DR35 GTGTCTGCAGTAATTGTCCACCTG

217. SEQ ID NO:219:DR36 GTGTCTGCAGTAATTGTCCACCC

218. SEQ ID NO:220:DR37 ATGTCTGCAGTAGGTGC

219. SEQ ID NO:221:DR38 CTCTCCACCAACCCGTAGTTGTA

220. SEQ ID NO:222:DR39 TGCACTGTGAAGCTCTCAC

221. SEQ ID NO:223:DR40 CTGCACTGTGAAGCTCTCCA

222. SEQ ID NO:224:DR41 CCCCGTAGTTGTGTCTGCAA

223. SEQ ID NO:225:DR42 GCAGTAGGTGTCCACCGC

224. SEQ ID NO:226:DR43 GCAATAGGTGTCCACCTC

225. SEQ ID NO:227:DR44 CCTTCTGGCTGTTCCCAGTG

226. SEQ ID NO:228:DR45 TCCTTCTGGCTGTTCCAGG

227. SEQ ID NO:229:DR46 ACAGTGAAGCTCTCCACAG

228. SEQ ID NO:230:DR47 CTCCGTCACCGCCCGGA

229. SEQ ID NO:231:DR48 CTCCGTCACCGCCCGGTA

230. SEQ ID NO:232:DR49 CTCCTCCTGGTTATGGAACTG

231. SEQ ID NO:233:DR50 CTCCTCCTGGTTATGGAAGTA

232. SEQ ID NO:234:DR51 TCGCTGTCGAAGCGCACGTCG

233. SEQ ID NO:235:DR52 CGCTGTCGAAGCGCAACGGAT

234. SEQ ID NO:236:DR53 CGCTGTCGAAGCGCACGTCG

235. SEQ ID NO:237:DR54 TCGCTGTCGAAGCGCAGGA

236. SEQ ID NO:238:DR55	TCGCTGTCGAAGCGCACGA
237. SEQ ID NO:239:DR56	ACGTCGCTGTCGAAGCGCAG
238. SEQ ID NO:240:DR57	TCACCGCCCGGTACTCCCT
239. SEQ ID NO:241:DR58	CCAAGCTCCGTCACCGCCT
240. SEQ ID NO:242:DR59	CCGCCCCAGCTCCGTCG
241. SEQ ID NO:243:DR60	GCTGTTCCAGTGCTCCGCAG
242. SEQ ID NO:244:DR61	GCTGTTCCAGTGCTCCGCAT
243. SEQ ID NO:245:DR62	GGCTGTTCCAGTACTCAGCG
244. SEQ ID NO:246:DR63	GCTGTTCCAGTACTCGGCGA
245. SEQ ID NO:247:DR64	TTCTGGCTGTTCCAGTACTCA
246. SEQ ID NO:248:DR65	CCGCCTCTGCTCCAGGAG
247. SEQ ID NO:249:DR66	CCGCGCCTGCTCCAGGAT
248. SEQ ID NO:250:DR67	ACCGCGGCGCGCCTGTCT
249. SEQ ID NO:251:DR68	CCGCGGCCCGCGCCTGC
250. SEQ ID NO:252:DR69	CACCGCGGCGCGCCTGTT
251. SEQ ID NO:253:DR70	CACCTCGGCCCGCCTCC
252. SEQ ID NO:254:DR71	GTCCACCGCGGCGCGCGT
253. SEQ ID NO:255:DR72	TGTCCACCGCGGCCCGCT
254. SEQ ID NO:256:DR73	TCCACCGCGGCCCGCGC
255. SEQ ID NO:257:DR74	TCCACCGCGGCCCGCTC
256. SEQ ID NO:258:DR75	TGTCCACCGCGGCCCGCT
257. SEQ ID NO:259:DR76	TAGGTGTCCACCGCGGCG
258. SEQ ID NO:260:DR77	GCGCCACCTGTGGATGACG
259. SEQ ID NO:261:DR78	TCTGCAGTAATTGTCCACCTG
260. SEQ ID NO:262:DR79	GTCTGCAATAGGTGTCCACCT
261. SEQ ID NO:263:DR80	CTGCAGTAGTTGTCCACCCG
262. SEQ ID NO:264:DR81	CCGTAGTTGTATCTGCAGTAGT
263. SEQ ID NO:265:DR82	CCGTAGTTGTGTCTGCAGTAGT
264. SEQ ID NO:266:DR83	CCCGTAGTTGTGTCTGCAGTAAT
265. SEQ ID NO:267:DR84	CCCGTAGTTGTGTCTGCACAC
266. SEQ ID NO:268:DR85	CAGCACGTTTCTTGGAGCTGT
267. SEQ ID NO:269:DR86	TTCTTGTGGCAGCTTAAGTTTGA
268. SEQ ID NO:270:DPA-E(PC)	GATCCCCCTGAGGTGACCGTG
269. SEQ ID NO:271:DPA-F(PC)	CTGGGCCCGGGGGTCATGGCC

270. SEQ ID NO:272:DQCPT1 CACGTCGCTGTCGAAGCGCACGTACTCCTC

271. SEQ ID NO:273:DQCPT2 CACGTCGCTGTCGAAGCGGACGATCTCCTT

272. SEQ ID NO:274:DQCPT3 CACGTCGCTGTCGAAGCGTGCGTACTCCTC

273. SEQ ID NO:275:DQCPT4 CACGTCGCTGTCGAAGCGCGCGTACTCCTC

274. SEQ ID NO:276:DQCPT5 CACGTCGCTGTCGAAGCGCACGTCCTCCTC

275. SEQ ID NO:277:DRCPT1 DRCP TGGCGTGGGCGAGGCAGGGTAACTTCTTTA

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